

Spacelab a monument to the past

The European spacecraft is neither an outright waste of money nor a potential platform for better things but a reminder that software (in the sense of forethought) is more important than mere hardware.

THE postponement of the first Spacelab launch until either November or next February is both a cruel disappointment for the European Space Agency and a salutary reminder of the dangers in this field, as in others, of putting too many eggs in one basket. Spacelab has been the focus of the space agency's forward planning since 1969, when the United States set out to interest potential partners in the development and operation of the space shuttle system. The chances were never bright that some other government would agree to become a minority shareholder in the shuttle as such. As events have shown, that would have been a blank-cheque commitment. More than a decade ago, it seemed safer that Europe should instead shoulder responsibility for a significant piece of hardware that would also make a substantial contribution to general understanding.

That is the origin of Spacelab, a removable vacuum-tight canister fitting in the cargo bay of the shuttle on whose development and construction the European Space Agency has spent more than \$1,000 million. The first flies in the ointment became apparent more than five years ago, when it became plain that the shuttle itself would be seriously delayed. Earlier this year, it was also apparent that the communications system needed to relay the full range of Spacelab data to the ground would be incomplete. Now the first flight of Spacelab is likely to be put off until next year, with all the financial consequences that will have for the space agency's budget. The agency's officials are doing the best they can to wring some kind of compensation from the shuttle's managers, the National Aeronautics and Space Administration in the United States. The people who have built equipment for the flight, and who must now kick their heels, are unlikely to be much comforted by whatever emerges from that negotiation.

But why blame bad luck? That will be the passably forgivable reaction of those responsible for this project. And it is true that in a different world, one in which ceramic tiles never peel off the shuttle's surface and in which rocket engines never malfunction, that line of defence would be acceptable. In the real world, however, the excuse carries no conviction. The trouble with the Spacelab project is that it has been from the outset hardware-led. The huge canister designed to occupy the shuttle's cargo bay is almost an embarrassment. Making the fullest use of the space available has meant that a miscellaneous collection of experiments, each thoroughly worthwhile in itself, has to be welded into a manageable package — and then equipped (or half-equipped) with a purpose-built communications system. The crew who will be looking after this motley collection of equipment next month or in February will have their work cut out to know what next to do.

Ambition

The underlying error is that the European Space Agency's ambitions more than a decade ago surpassed what should have been its realistic assessment of its members' needs. Even then, when the decision to embark on Spacelab was taken, Europe was filled with groups from several disciplines clamouring for the opportunity to launch instruments into orbits about the Earth. With the passage of time, the clamour has become louder. But most of the unsatisfied users of orbits about the Earth would prefer that the effort spent in designing and developing an instrument should be requited by a spell of several months, even several years, in orbit —

not just the few days that the shuttle will provide at this early stage. Sensibly enough, the European agency is hoping to develop a container that will be roughly half the size of Spacelab (rejoicing in the name EURECA) which would be launched on a commercial basis by the shuttle, and which could be left in orbit for several months before recovery and reuse. That could be a means of satisfying the need that has arisen. The hope now is that the agency's enthusiasm for Spacelab will not have persuaded member governments that the agency is not to be trusted.

Meanwhile, it seems certain that Europe will become the chief source of published information on the behaviour of materials in circumstances in which gravitational forces are only a small fraction — one thousandth or even a ten-thousandth — of those on the surface of the Earth. From the outset, the European agency has made "microgravity" its own. The result is that the first version of Spacelab has been fitted out with a variety of equipment that should throw important light on such things as the form of the interaction between different phases of the same material, or the behaviour of composite materials in circumstances in which the different densities of different components are of no account. Most of the experiments planned for the now postponed launching of the first Spacelab will be valuable contributions to basic understanding. As yet, however, it seems to be agreed among those responsible for these experiments, it is too soon to know whether it will ever be worthwhile to use orbiting stations as miniature factories for the production of superior alloys, or of pharmaceutical products of much greater purity than can be had on the surface of the Earth. The snag, unfortunately, is that by no stretch of the imagination would the member states of the European Space Agency have clubbed together to mount such an investigation if it were an isolated objective. Even the microgravity programme, imaginative and potentially valuable though it is, is in the last resort nothing but a kind of space-filler in almost the literal sense.

Neglect

The moral in all this should be clear. More than ten years ago, the European Space Agency (or, more strictly, the European Space Research Organization from which it partly sprang) seriously misjudged its future role. Impressed with the need for a project large enough to serve as a focus for a whole range of activities, and anxious also to demonstrate that Europe could play a part in partnership even with organizations such as the US National Aeronautics and Space Administration, the managers of this nascent activity plumped for hardware when software — or even nothing — would have better served the need. For much of the intervening period, they and their successors have been criticized for their neglect of basic science, especially when the development of the Ariane rocket system was eating holes in the collective budget. The danger now is that past errors will be taken as proof of present fallibility — a danger all the greater because the space agency's account of what Spacelab will accomplish when eventually it is launched shows no trace of the self-doubt that would be proper. To be safe, however, what the agency should be saying is that Spacelab is a monument to an earlier period — one in which governments were more prosperous and the technical community less certain (for good reason) what might be done with Earth satellites.



Double-talk at USDA

The US Government must be both resolute and guileful to make sense of agricultural research.

THE remarkable thing about the failure of the US Department of Agriculture (USDA) to change the way it supports research is that nobody will come right out against competitive research grants. Representative Jamie Whitten, chairman of the House Appropriations Committee, member of Congress for 43 years and staunch defender of the time-honoured practice of doling out research funds to each state's land-grant university regardless of scientific merit (a practice that not coincidentally props up research institutions in Mr Whitten's home state of Mississippi), swears he is not against competitive grants — even as his committee cuts the heart out of that already enfeebled programme. If Mr Whitten gets his way, it will be \$7 million poorer than last year. (It has never crept above \$17 million, a dwarf by comparison with the almost \$2,000 million that the federal government and the states spend altogether on agricultural research.) The directors of the state experiment stations at the land-grant universities also swear they are not against competitive grants — even as they stage a campaign to force out the White House official who dared to convene a workshop on agricultural research and dared to conclude that any new research funds should go to competitive grants. And, now, the new administrator of USDA's Cooperative State Research Service, the agency that administers the land-grants' research funds, says that he, too, is not against competitive grants. Only he, for one, is not dissembling.

Indeed, there is good reason to take Dr John P. Jordan at his word when he says much more money is needed for competitive peer-reviewed research grants for agriculture. Dr Jordan was most recently director of the Colorado experiment station. He is a PhD biochemist whose own laboratory depended heavily on competitive grants from outside USDA (both the National Science Foundation and the National Institutes of Health support more basic plant molecular biology research than does USDA). Thus he is well able to appreciate the changes that have lately taken place (not for the better) in agricultural research. "The storehouse of basic knowledge has been depleted", he says, and competitive, peer-reviewed grants will have a big part to play in restoring scientific expertise and prestige to agriculture. Dr Jordan sees his own appointment as a sign that USDA is serious about science and about increasing support for competitive grants. This, together with the establishment of a separate office for competitive grants and the creation of an assistant secretary of agriculture in charge of science, have, he says, "set the stage" for change.

To circumvent entrenched interests, however, USDA will need to do more. A polite request in the President's 1985 budget and reassurances from Dr Jordan that new funding for competitive grants will not undermine the base of support to the land-grant universities are not going to soften Jamie Whitten or his constituency of frankly uncompetitive south-eastern universities. If USDA is serious about change, it will have to fight an all-out war on two fronts, one ideological, one pragmatic.

The ideological front will be the harder of the two for the department because it is unfamiliar terrain. Simply, USDA must cease to present science as something separate from agricultural policy as a whole. In a world that has perhaps forty years in which to double food production, agricultural research is much more than something to provide a better standard of living for farmers. And with the fruits of conventional applied agricultural research drying up, partly because there is only so much that better use of fertilizer can accomplish, the need to replenish the basic store of knowledge is ever greater. We have done everything we can with applied research; we now have to tackle the organism itself.

A basic understanding of plant molecular biology, and its application in genetic engineering, will also yield solutions of the main problem that preoccupies USDA, how to keep farmers in business. Genetic engineering holds the promise of reducing input costs while improving yield, thus easing the pressures that force farmers to expand to maintain income, plant fencerow to

fencerow, till marginal and erodible land and produce ever-greater local surpluses that only increase the cost of price supports.

On the pragmatic front, USDA — and research scientists — must play the political game. They should take a lesson from the director of USDA's in-house Agricultural Research Service (ARS), who has succeeded, against all political odds, in closing six of its most inefficient field laboratories. ARS, no less than the land-grant funds, has been the victim of congressional politics based on the principle of bringing home the goodies for the home district. Many of its regional laboratories are inefficient, under-utilized or directed to outdated problems, none of which worries the congressmen in whose district they lie. ARS's administrator, Dr Terry Kinney, has worked with local politicians and industry in what should be an obvious approach, but unfortunately is not: he combines reassurances that any money saved through closing a laboratory will remain in the region with an unabashed effort to find a local constituency that will back a new research initiative. A congressman faced with competing demands from two separate constituencies is sure to look deeper into their relative merits than one faced with a single constituency fighting the loss of some prize for which he himself fought long and hard.

USDA, if it is serious about competitive grants, can do the same with the scientific community. Many states — particularly the larger ones — have first-rate land-grant universities that would benefit from increased peer-reviewed support for agricultural research; private universities can make a legitimate claim on their state's elected representatives as well. It is time for USDA to put its not inconsiderable weight where its mouth is. □

Set graduates free

Reform of British public support for graduate students is overdue; a chance has arisen.

AMONG systems of higher education, the British stands out in one striking respect — a large proportion of those reading for higher degrees are supported financially by the government. Over the decades since the Second World War, this has helped to increase the output of doctoral graduates, especially in the sciences. For most of that time, there has been general applause for the way in which public support for graduate students has helped to sustain the volume of research as such and to ensure that those drawn into this expanding enterprise include some of the most able. Only recently has the system attracted criticism of importance — first, three years ago, the suspicion based on performance at different universities that many graduate students embark too lightly on a research career and then too casually drop out, or that many graduate studentships are too often mistaken for lifelong careers. More recently, as funds have shrunk, more particular complaints have arisen. Geophysicists at the universities have, for example, taken to complaining that too few studentships are awarded to young people in that field. This is one of the issues certain to be provoked by the invitation from the Natural Environment Research Council for comments on its handling of studentships (see page 661).

It must be hoped that comments will be more radical than that. The majority of graduate studentships are at present awarded on a quota system, so many for particular university departments, so many (fewer) for others. Although the Science and Engineering Research Council has a kind of reserve by means of which bright people unlucky in the competition for allotted places can be rescued, there can be no assurance that the system is as equitable as it is intended to be, while the pattern of research followed by graduate students is determined less by their own interests than by the decisions of the research councils. The remedy will not be found by a different distribution of studentships but only by more radical revisions of the system. Abolition of the quota system altogether would be better. Allowing would-be graduate students some say in which universities should have how many of them would be another step towards the pattern of diversity which most of those in charge now believe should be encouraged. □

Argentine nuclear plans

US blunders on heavy water transfer

Washington

A LOOPHOLE in the Nuclear Non-Proliferation Act has allowed the Reagan Administration to approve the transfer of 143 tonnes of heavy water to Argentina despite indications that that country is acquiring a nuclear weapons capability. As a result, efforts are being made in Congress to tighten export controls on all nuclear material.

Argentina, which possesses substantial uranium reserves, will soon be self-sufficient in fuel fabrication and reprocessing. It already has its own uranium milling and fuel fabrication plants, and a conversion plant (where uranium ore is processed into fuel-grade uranium dioxide) supplied by West Germany and it has under construction its own conversion and reprocessing plants and a heavy water plant supplied from Switzerland. Heavy water is used as a moderator in reactors that use natural uranium fuel (0.7 per cent uranium). Argentina has two commercial reactors of the Canadian CANDU type.

There have recently been intelligence reports suggesting that Argentina is planning to divert as much as one tonne of uranium at the conversion stage without detection by international inspectors. Although Argentina has no uranium enrichment facilities and does not import enriched fuel that could be used directly to construct a bomb, the unenriched fuel could be irradiated in its reactors to produce plutonium, which could then be separated at the reprocessing stage for weapons use.

The sale of heavy water by the United States was approved because of a loophole that allowed the administration to circumvent the Nuclear Regulatory Commission (NRC), which must approve most transfers of nuclear fuel or components, and which is autonomous and politically independent of the executive branch. In fact, several requests for direct sales of relatively minute amounts of heavy water — 5 to 10 kilograms — to Argentina have been held up by NRC for more than a year. NRC, before approving a sale, must ensure that the country receiving the material will maintain international inspections at the site to which the material is going and pledge that that site will not be used to produce nuclear explosives.

The 143-tonne shipment, however, is technically a "retransfer", not a sale; the material, although manufactured in the United States, is in two West German reactors scheduled for decommissioning. Retransfers need only the approval of the Secretary of Energy, a political appointee.

At recent hearings on the matter, several astonished senators heard the State

Department's ambassador-at-large for non-proliferation policy, Richard Kennedy, explain that the administration had "goofed" in not consulting Congress before approving the retransfer. Kennedy also said that the administration was satisfied that Argentina's military interest in nuclear energy was limited to propulsion for its submarines. Argentina has refused, however, to rule out the development of

nuclear explosives for "peaceful" uses.

The House of Representatives has passed an amendment to the Export Administration Act that would block both the Argentina deal and the sale, announced this summer, of spare parts for India's Tarapur reactor. The amendment requires that a strict standard, now applied only to the sale of nuclear fuel, be applied to the sale and retransfer of all nuclear materials and technological assistance: to receive such aid, a country would have to open *all* its nuclear facilities to international inspection and would have to pledge that it will not seek to build nuclear explosives. Similar measures are pending in the Senate.

Stephen Budiansky

US conservation

What will happen after Watt?

Washington

FOR all the sound and fury of James Watt's controversial reign as Secretary of the Interior, which ended much as expected last week with his resignation, he in fact accomplished few of his overt objectives. The irony of his enforced resignation is that he was in the end brought down not by a dispute over the disposal of federal lands, on which his policies have repeatedly maddened conservationists, but by a tasteless jibe that offended ethnic minorities, women and the disabled.

The surprise selection of William Clark, the President's national security adviser and close friend, to succeed Watt, however, is a sign that Watt's less-noticed accomplishments, particularly his ability to bring political rule to traditionally independent sections of the department, will live on after him. In choosing Clark, Presi-

dent Reagan took the opposite tack from that he followed in bringing in William Ruckelshaus, a respected professional, to clean up the disorder left by Anne Gorsuch at the Environmental Protection Agency. Clark is in the same western-state conservative mould as Watt, and his selection is seen as a concession to the conservative wing of Reagan's party.

Watt's most controversial policies of leasing mineral rights on previously protected federal lands and coastal areas have been largely quashed by congressional action and undercut by a lack of interest on the part of the oil companies in leasing new fields at a time of over-supply and low prices. Much the same happened when Watt tried, two years ago, to sell leases on oil-shale territories in Colorado and other western states — potential bidders were reluctant to risk their own money on an unproven technology. Watt's efforts to accelerate the lease of offshore oil rights ran into restraining orders issued by federal judges in response to lawsuits by states with competing interests — mainly fishing.

Watt's greatest legacy, however, may be in the less obvious organizational changes he effected within the department. Congressional committees found repeated instances in which professionals in the department who questioned the legality or propriety of Watt's policies were transferred or harassed. By many accounts, he was thoroughly successful in eliminating the conservation interests in the department, a body of expertise that may take years to rebuild. He also brought political control within the department to a new peak by ordering professionals, including the superintendents of national parks, not to testify before congressional investigating committees.

Even the staunchly independent US Geological Survey did not escape; its conservation division, including a number of scientific personnel, was subsumed by a new Minerals Management Service, under the control of a political appointee.

Stephen Budiansky



In April President Reagan awarded James Watt a model foot with a hole in it to celebrate Watt's achievement in "shooting himself in the foot" by banning the Beach Boys rock group from Washington's Fourth of July celebrations. Watt's latest blunder was rewarded more severely.

European research

SERC struggles to pay

BRITISH research councils may next year be spared some of the financial difficulties arising from the recent fall in the value of sterling and the consequent increased cost of subscriptions to international organizations. A committee set up by the Treasury is understood to have recommended some alleviation of the financial embarrassment likely particularly to afflict the Science and Engineering Research Council (SERC) in the financial year 1984–85, beginning next April.

The final outcome will not, however, be known until next month, when the Advisory Board for the Research Councils (ABRC) will be making its annual recommendation on the distribution of the UK science budget between the five research councils and smaller organizations such as the Royal Society. Meanwhile, within SERC itself, a manpower review panel has been set up, stimulated partly by cash shortages but also by the Rayner review of

its operations, to decide whether the number of SERC research institutes should be reduced.

The fall in the value of sterling and the rise in the UK gross domestic product relative to that of other member states mean that international subscriptions to the European Organization for Nuclear Research (CERN) and the European Space Agency (ESA) will cost SERC an extra £10 million or so. The problem became acute earlier this year (see *Nature* 303, 742; 1983) when the council's research boards were required to consider several drastic options. Meanwhile, a committee under the chairmanship of Miss J. Kelley was set up to consider whether there might be scope for relaxing the rigid accounting rules applied by the Treasury to public spending and which, for example, do not allow surplus credit to be carried over from one year to another.

In the past, SERC has sometimes found itself embarrassingly flush with funds towards the end of a financial year and has encouraged hasty applications from the universities to ensure that no surplus is left. But this year the currency situation has left the council with a cash shortage which has resulted in various drastic measures such as a temporary halt on grant payments by the Astronomy, Space and Radio (ASR) Board.

The Kelley committee has now reported to its ministers, although its advice and even its membership is classified as secret. The Treasury is, however, believed to have conceded ground to the case for extra flexibility. Although action has still to be decided, it seems that a bid by ABRC earlier this year for supplementary funds for SERC has fallen on fertile ground.

Nevertheless, SERC is unlikely to find that all its problems melt away. The Nuclear Physics Board, whose particle physics research depends on the council's subscription to CERN, is already under pressure because of the size of this contribution. Despite CERN's leadership in the accelerator league, the *per capita* cost of high-energy physics remains high and the side-benefits small or at least disputed. But the international nature of the project, current excitement about new particles and the apparent personal interest of the Prime Minister make CERN a tough target for cost-cutters.

The Science Board (responsible for such fields as chemistry, solid-state physics and some aspects of biology) has also been undergoing an internal review. By highlighting areas of research requiring encouragement, it is becoming more directive than reactive. One consequence may be that important projects are protected even if SERC follows precedent in distributing budgetary shortages equally across all research boards.

Philip Campbell

French research policy

Fabius looks to Europe

Paris

CAN socialist France influence conservative Britain and West Germany to cooperate on science and the new technologies? Only time will tell, but the next few months will certainly see an intense French diplomatic effort to create what Laurent Fabius, French minister of state for industry and research, calls a "European space" for science and technology. One sign of this was the bullish memorandum submitted to the European council of ministers earlier this month (see *Nature* 6 October, p.462), to be discussed at the summit in Athens in December.

Fabius outlined his views on the French position on science and technology earlier this month. Science and technology, he says, have to be seen in relationship to industry; and while the individual nations of Europe have made "great efforts" in each of these areas, "none is big enough", and there is duplication and waste of effort. France seeks two things in research and industry in Europe: better coordination and common projects. These are separate ideas, Fabius emphasized. "If the nations continue to act apart, they risk being overtaken technologically and scientifically", he said.

But is not France rather alone among the bigger European countries in its commitment to research and in its devotion to central planning? Can French strategies really take root in the rest of Europe? Fabius thinks they can. "I don't know if the differences are so great", he said. "Ideology is one thing; when you have power you have to face facts", said Fabius, breaking into English. "Above all, there are financial constraints and, then, we don't have any central planning at all. We think it is good to have a sort of strategic approach — to define a few priorities (not too many) and to associate everyone in the effecting of these priorities: industry, laboratories, public and private finance. . . . Yes, we are making a big financial effort, because we think that research is the key to the future: a modern economy needs flexibility and research gives us that. Research is fundamentally what separates a strategy of hope from one of resignation."

But "when I talk to my opposite numbers in Germany, the United Kingdom and others, political colour doesn't enter at all". Other countries in Europe, said Fabius, "have the same concern [for research] but it is more difficult for them to have a very activist attitude — that's the difference". Fabius, who visited Britain a few weeks ago for discussions at the Departments of Trade and Industry and Education and Science, considers that "there is a big effort (though certainly in

Observatory at risk

THE Astronomy, Space and Radio (ASR) Board, faced with the possible need to find another £1 million for international subscriptions out of its £40 million budget for 1984–85, has set up a manpower advisory panel to examine the staffing and roles of three SERC institutes, the Rutherford Appleton Laboratory, the Royal Greenwich Observatory (RGO) and the Royal Observatory Edinburgh. Under the chairmanship of Professor A. P. Willmore, an X-ray astronomer at the University of Birmingham, the committee will in particular consider whether all three institutes are necessary.

On the surface, RGO seems to be the most vulnerable because it is in a process of transition towards becoming a centre for the remote use and control of new telescopes (currently being developed at La Palma in the Canary Islands) via satellite links. Moreover, a recent review by the government's efficiency body (the Rayner unit) has recommended the sale of Herstmonceux Castle — a substantial part of RGO's facilities (see *Nature* 304, 3; 1983). However, recently developed detectors are enabling British ground-based astronomers (including those at the two royal observatories) to maintain research programmes that are internationally competitive and it is by no means a foregone conclusion that the manpower panel, which will report next January, will opt for change. An old chestnut of an argument (some might call it a conker) underlying its discussions relates to the proper balance between research carried out in universities and that carried out in SERC institutions.

Philip Campbell

different ways) in Britain, an important one I think, and also in Germany, and there's a wish in other countries too. But it's certain that the effort is strongest and most coherent in France".

On European cooperation, Fabius considers plans for the coordination of research in electronics, informatics and so on to be "good enough". The concrete consequence, the Brussels-inspired Esprit programme for research and development in new information technologies, "has the support of all the countries of Europe". There are a few "nuances" distinguishing the countries over methods of running the programme (for example, there are fears of creating a new bureaucracy) and "some financial problems", but on the fundamentals of the programme, there is agreement that it should devote "a great amount of resources" (1,500 million ECU) and that while Brussels will pay 50 per cent, industry must also pay 50 per cent "to guarantee involvement". Esprit, says Fabius, will concentrate on the "technology of the future".

Cooperation in big scientific equipment (as at the European Organization for Nuclear Research at Geneva) is already successful and may be extended. In political coordination "there is a favourable trend" and in common programmes "there are some very positive things" (such as Esprit). "But Europe, including France, does not want these programmes to be directed against anyone — they are for the countries of Europe. And since all countries are suffering from financial constraints, the question is more one of the better coordination of what exists than of raising massive new expenditures. In industry, things are more

delicate; but in research and technology, very favourable."

What are the main problems facing his European policies? "First, national egotism and traditions; second, the fear in many countries of bureaucracy, of rigidity and of *dirigisme*. We must take care that this is not what happens, that we coordinate action with the governments retaining control and, above all, that we do not create jobs for civil servants."

The third obstacle is financial. "Financial constraints tend to induce governments to sacrifice the long term, but this is just the time when a government must emphasize the long term."

Fabius is also greatly interested in the opening-up of public markets in Europe (for example in telecommunications). "To say that we have European collaboration when public markets for high technology products stay completely closed is a contradiction that cannot be maintained for ever", said Fabius. "We must prepare for a certain reciprocity; I think this is one of the great directions of development."

As no doubt he might. Whatever the logic of his views in general, the French electronics and telecommunications industry, the leading edge of the French Government's drive into the twenty-first century, is desperately in need of new markets.

So did Fabius, when recently in London, discuss the forthcoming privatization of British Telecom (the nationalized British telecommunications monopoly)? "No, we discussed the opening of markets." And what was the reply? Fabius laughed. "The response was much faster on privatization than on opening markets. . .".

Robert Walgate

dispute at next week's meeting.

Columbia refused last week to comment on the AAU proposal but confirmed that it would continue to employ the Washington lobbying firm, Schlossberg-Cassidy and Associates, which masterminded its congressional campaign last May. Columbia's president, Michael Sovern, is known to believe that the university did nothing different in kind from the tactics regularly used by other major universities in promoting their interests in Congress.

The president of Catholic University, Father William Byron, is equally unrepentant. He maintained last week that it would be inappropriate for AAU, a voluntary membership body, to issue a statement that sought to regulate the activities of individual universities. Any statement AAU did formulate, he added, should draw a careful distinction between research programmes — which ought to be peer reviewed — and funds for buildings to house them. All the money Catholic and Columbia gained in May was for buildings.

A number of AAU members regard the distinction as casuistical. And they insist that the manoeuvres of Catholic and Columbia were an unusually brazen use of pork-barrel politics by institutions that should know better. Catholic's funds emerged only after the university mobilized the bishops among its trustees to press the merits of the vitreous state laboratory on their local congressmen. House speaker Tip O'Neill intervened personally to support the budget amendments.

According to Father Byron, the university would never have considered asking for the extra funds but for the controversy surrounding the White House proposal for NCAM, which had itself been rushed into the federal budget at the last moment and, in the view of many materials scientists, without having undergone adequate scientific review. While agreeing to provide funds for Catholic and Columbia, Congress chose to be high-minded about the absence of peer review for NCAM, and reduced the \$26 million White House request to a mere \$3 million.

Since then, Dr Keyworth has been forced to watch the NCAM proposal unravel still further. A Department of Energy panel, chaired by Albert Narath of Sandia Laboratories, has produced a report that questions the chief idea behind NCAM — that materials research at Berkeley should be coupled in a single facility with an advanced new synchrotron light source.

According to the Narath panel, the materials research centre and the light source projects should be treated separately because the latter would constitute such a large undertaking that it could be expected to unbalance the activities of a single centre for materials research. The panel does recommend pressing ahead with Lawrence Berkeley Laboratory's plans for a new center but suggests calling it the Berkeley Center for Advanced Materials to reflect its more modest role. **Peter David**

US universities

Academic lobby over pork barrel

Washington

AMERICAN universities are squaring up for what promises to be a bad-tempered argument about the way they compete for federal research funds. At a closed meeting in Los Angeles next week, presidents of the 50 elite research universities belonging to the Association of American Universities (AAU) will decide whether to reprimand two of their number for using congressional sleight of hand to win federal support for projects that would not otherwise have been funded.

The two universities, Columbia University in New York and Catholic University in Washington, caused uproar last May when they hired a professional lobbying firm to talk Congress into spending money on two projects that had not been reviewed by the federal science bureaucracy or routed through the specialist science committees (see *Nature* 303, 272; 1983). Catholic received \$5 million to start building a new vitreous state laboratory and Columbia received \$5 million for new

chemistry facilities.

Despite the small sums involved, the manoeuvre outraged both the White House and a number of fellow universities. The \$5 million for Catholic's vitreous state laboratory was poached from the \$26 million requested by presidential science adviser George Keyworth for the proposed National Center for Advanced Materials (NCAM) at Lawrence Berkeley Laboratory. Columbia's \$5 million was cobbled together by raiding funds earmarked for upgrading Van de Graaff accelerators at Yale and Washington Universities and for other university instrumentation projects.

Many university presidents have remained angry enough to insist that AAU issue a statement on the affair, calling on members to curtail their individual lobbying efforts in Congress and to reaffirm the principle that scientific merit, not political clout, should determine the allocation of federal research funds. But the proposal is certain to provoke a fierce

Spacelab launch

Last-minute hitch delays shuttle

Washington

THE much-delayed maiden flight of Europe's Spacelab has been postponed again. Spacelab was to have been flown aboard the space shuttle Columbia on 28 October, but the National Aeronautics and Space Administration (NASA) will not mount the flight until it has discovered the reasons for a malfunction in the shuttle's solid rocket boosters.

The reusable boosters help the shuttle into orbit and are later dropped into the sea and recovered for future missions. Examination of one of the rockets used by Challenger last August revealed that in one of the two boosters, the lining of a nozzle had burned away to within two-tenths of an inch of the metal surface. Had the rocket fired for longer it could have burned a hole in the nozzle, with potentially disastrous consequences for the shuttle.

Early last week, NASA believed that it had traced the cause of the trouble to the use of a defective batch of lining material in the boosters. This theory was supported when a test firing on the ground, using lining material from the same batch, resulted in an identical malfunction. But on Wednesday a second test firing, again using the same batch of lining, did not malfunction.

By the end of last week, NASA officials conceded that there was no hope of identifying and remedying the cause of the problem in time for the 28 October launch date. Spacelab's masters now face an unpalatable choice between postponing the mission until next February or trying for a launch at the end of next month, by which time Spacelab would have to spend more hours in daylight, thereby degrading the scientific return from some of its astronomical and physics experiments.

At a meeting in Houston last week mission scientists were reported to be divided, with the life and materials scientists pressing for an early launch while the astronomers argued for a delay until next year. The European Space Agency (ESA) said the delay will cost up to \$400,000 a month, and is asking NASA to guarantee that any experiments degraded as a result of the postponement will be allowed to fly free of charge on later Spacelab missions.

The yield from the scientific experiments carried aboard Spacelab has already suffered as a result of NASA's recent mishaps. Spacelab's maiden voyage, planned for last September, was delayed because the first of two new satellites designed to provide communications support failed to reach its proper geosynchronous orbit in time. After being placed in an elliptical orbit, the Tracking and Data Relay Satellite (TDRS) was carefully nudged into its correct orbit and is now functioning well. But NASA will not launch the second TDRS until it is sure that

the problem will not recur.

Spacelab mission scientists decided that the absence of a second TDRS was acceptable, but several experiments will yield small returns because the instantaneous relay of data and instructions between ground and the spacecraft instruments will not be possible as originally

European space science

Two more projects planned

Two new European space science missions, one on solar physics including oscillations (SOHO), the other on plasma physics (Cluster), are likely to go ahead for detailed "phase-A" studies following a recommendation by the European Space Agency (ESA) space science advisory committee last week. The meeting at Frascati, Italy, was enlivened by a 5-minute add-on presentation by French solar physicist Philippe Delache, who tentatively announced his discovery of gravitational waves absorbed by the Sun (see p.665). Did the announcement influence the committee's decision? "It helped", said one observer, "but it was by no means decisive, SOHO would have been approved anyway."

Before the committee (which is advisory to the governmental Science Programme Committee, meeting in November) were five proposals: SOHO, Cluster, AGORA (a multiple asteroid rendezvous), FIRST (infrared telescope) and XMM (X-ray multiple mirror).

AGORA at around \$340 million would be "relatively expensive" by ESA standards, said a spokesman (ESA's science budget is \$110 million per year), and required an ion-drive engine to rendezvous with and orbit around 3 or 4 main belt asteroids. The committee decided that it was too early to back this project but that the development of ion-engines (abandoned in the United States) should be helped along in Europe and collaboration over AGORA sought with the National Aeronautics and Space Administration.

FIRST and the XMM were also considered to be "ambitious", requiring new technology which should be developed further before going to phase-A, the committee recommended. But the committee did recommend that SOHO and Cluster should progress to phase A. SOHO, estimated to cost some \$200 million, is designed for the study of solar oscillations, the corona and the solar wind from the L_1 Earth-Sun lagrangian point, 1.5 million km from the Earth. Cluster (also about \$200 million) would be a group of four satellites in a close tetrahedral array, in an eccentric orbit around the Earth, designed to perform three-dimensional experiments and observations on space plasmas.

planned. For ESA, which has already spent more than \$1,000 million on Spacelab, the latest delay is a serious disappointment, but it is NASA that stands to lose most. The agency is trying to persuade the President to let it build a space station, but is being opposed by the Department of Defense, which wants the shuttle perfected first. The succession of delays in shuttle launchings will hardly strengthen NASA's argument that the shuttle is working well enough already.

Peter David

Assuming the committee's recommendations are accepted by ESA member states in November, the next decision point for these projects should come in mid-1985, when the phase-A studies are complete. But it will not then be a simple choice between SOHO and Cluster, for the committee also recommended last week that Kepler (a Mars orbiter) and X-80 (X-ray mission), which only just failed to be accepted for the next ESA space science launch (1992, when ISO, the International Solar Observatory will go up) should be updated and reviewed in conjunction with SOHO and Cluster at the appropriate time. The launch date for the lucky winner could be 1992 or shortly after.

Robert Walgate

Soviet space routine

THE two Soviet spacecraft, Venera 15 and 16, that went into orbit around Venus last week, appear to be a slight backward step in the Soviet planetological programme. Neither has a descent or landing stage, and they were designed for orbital missions only.

Somewhat unusually, this was announced at the time of launch. TASS said on 7 June that neither probe would have a landing stage, and that their programmes would consist of atmospheric studies (using infrared spectrometers made in East Germany) and the compilation of maps by radar. This unusual frankness may be related to the revelation in the same month (in two *Pravda* articles) that the Soviet Union now has launch facilities at Kapustin Yar and Plesetsk as well as at the well-publicized Baikonur landing site.

The dispatch of two Venera probes on a routine but scientifically valuable mission of this kind suggests that Soviet space planners no longer feel it necessary to attempt spectacular advances with every mission. Orbital stations, whether manned or unmanned, have long become a matter of routine. This does not mean, however, that planetary studies are about to lose their prestige value. The next scheduled pair of Venera probes will be used for the much publicized Vega mission, which will combine investigation of Venus with a penetration or fly-by of Halley's comet.

Vera Rich

UK energy conservation

New mood at ministry

THE British Department of Energy is not allowing its enthusiasm for nuclear power to overshadow its concern for energy conservation, and to prove the point it has financed a new booklet on energy conservation for distribution to schools throughout Britain.

The booklet, *Living with Energy*, is based on an exhibition produced by the Lothian Energy Group, a charity devoted to rational use of the Earth's resources. It describes measures that can be taken to

Development of combined heat and power schemes, as percentages of heat requirements

Denmark	25 per cent
Sweden	20 per cent
Finland	17 per cent
West Germany	8 per cent
The Netherlands	1 per cent
United Kingdom	1 per cent

Combined heat and power schemes are expanding in all the countries listed. From *Living with Energy* (Lothian Energy Group).

reduce energy waste in the home, and observes that national consumption could be reduced by improvements in the railway network. A pictorial feature on combined heat and power schemes is accompanied by a table showing that UK development of such schemes lags far behind that in several other European countries. On renewable energy sources, the booklet argues that fossil fuels should be preserved for essential purposes, while solar and other renewable energy sources are likely to become economically more attractive as fuel prices soar. Nuclear power, in contrast, scarcely gets a mention.

Government support for research into nuclear power does, of course, massively exceed that for renewables — but the new



Sir Sam Edwards, professor of physics at the University of Cambridge, has been appointed chief scientific adviser at the Department of Energy with effect from 17 October on a part-time basis.

Secretary of State for Energy, Mr Peter Walker, seems more inclined than his predecessor to take energy alternatives seriously. Mr Walker surprised many when, shortly after taking office, he publicly described Britain's energy conservation efforts as "apathetic". A new Energy Efficiency Office has been promised, and Mr Walker is expected to be an enthusiastic advocate.

Tim Beardsley

Graduate disputes

ACADEMICS, students and others in Britain who may have strong feelings about the way the Natural Environment Research Council (NERC) allocates its research studentships have been given an opportunity to air their views. The council has set up a committee under the chairmanship of Professor R. J. Berry, of University College, London, to review the workings of the system within the framework of drastic change in the universities.

NERC awards 300 research studentships a year, and supports graduate students in both universities and polytechnics. But the way grants are allocated has been the cause of some controversy. At issue is who decides what research areas are most deserving of support.

Before 1972, the council made quota awards to universities, which would then decide for themselves exactly how the money should be spent. The common complaint then was that many smaller universities and polytechnics were not awarded their fair share.

Under the present arrangements, NERC invites applications for specific projects from universities and polytechnics, each tied to a particular supervisor. Now the complaint is that host departments do not have enough control over what research will be done.

According to some dissatisfied potential accords, the projects supported simply reflect the interests of the members of NERC's training awards committees. If the departments concerned had more say, their argument goes, students would be allocated more equitably between supervisors and subjects by active researchers.

The committee will also be aware of the changing climate of opinion on how subjects for research studentships should be chosen. An influential report published earlier this year by the Advisory Council for Applied Research and Development recommended that research councils should become more selective. Berry's committee will report midway through next year, and its recommendations will be taken into account in the shareout of training awards for the academic year starting October 1985.

Tim Beardsley

Nuclear waste

Sludge sites to be sought

BRITISH Nuclear Fuels Ltd has announced that it is to spend £10 million on further reducing its discharges of plutonium and americium into the Irish Sea. The decision follows revised estimates of population dose which show that critical groups are still exposed to radiation levels which exceed recommendations for recurrent annual dose made by the National Radiological Protection Board (NRPB) and the International Commission on Radiological Protection (ICRP).

Most of BNFL's discharges come from its fuel reprocessing plant at Sellafield, formerly Windscale in Cumbria. The critical group, for the purposes of assessment, is eaters of fish and shellfish on the Cumbrian coast. The revised dose estimates were made after a reassessment of consumption patterns by the Ministry of Agriculture, Fisheries and Food and new data on gut uptake of actinides published by NRPB. The effect of the changes is to make actinides (especially plutonium) from molluscs the largest contribution to critical group dose, which in 1981 was 2.2 millisieverts per individual (effective whole body dose equivalent). The NRPB and ICRB limit for members of the public is 5 millisieverts with the recommendation that average annual dose should not exceed 1 millisievert for individuals exposed for prolonged periods. Average dose to the critical group was reduced to 1.5 millisieverts in 1982, and BNFL now expects a further reduction of this amount by a factor of five.

Discharges from Sellafield are already down to a fifth of the peak reached in the early 1970s, and with reductions now planned, the dose to the critical group will be only a few per cent of recommended limits.

Removal of waste from the discharge pipeline does not, of course, make it disappear. The result will be a concentrated sludge classified as intermediate level waste, which becomes the responsibility of NIREX, the Nuclear Industry Radioactive Waste Executive. An existing shallow land disposal site at Drigg is expected to be full before the end of the century, and NIREX will shortly announce two locations for further investigation as possible future land dumping sites. One of these will be a deep disposal site, probably a disused mine, suitable for long-lived intermediate level waste such as that from Sellafield. Several local authorities up and down the country which suspect that NIREX's preferred locations are on their territory are already preparing themselves for a fight over planning permission. However, as their number exceeds the number of sites NIREX has short-listed, many of them will be disappointed.

Tim Beardsley

UK animal welfare

Government plans upset RSPCA

THE Royal Society for the Prevention of Cruelty to Animals (RSPCA) has launched a broadside attack on the British Government's proposals for legislation on the use of animals in scientific procedures. According to RSPCA, the proposals, published in a white paper in May, are "sadly deficient in crucial areas" and "fail totally" to meet what it sees as the central issue, the control of pain and suffering.

To illustrate its misgivings, the society's Animal Experimentation Advisory Committee has compiled a 350-page dossier, *Pain and Suffering in Experimental Animals in the UK*, in which are reproduced 35 scientific papers, all published within the past eight years, which describe procedures that suggest suffering was caused to animals. According to Ms Sheila Silcock, research consultant to the committee, several of the procedures are remote from possible benefit to man or animal and some suffer from serious deficiencies in experimental design or in the model systems used.

Each is accompanied in the dossier by a detailed critique. The society had felt that the debate on animal experimentation was becoming too abstract, and wants the government to say whether the procedures described would be permitted or modified under the proposed new legislation. The society does not want any details of the papers to be given which could lead to their being identified, but they include experiments on trauma, therapeutic drugs and poisons. The document has now been submitted to the Home Secretary.

The government's proposals (see *Nature* 303, 391; 1983) include the continuation of the provision that experiments are not permitted which cause "severe and enduring" pain. While recognizing the inherent problems of definition, RSPCA says that in no circumstances should animals be subjected to pain that is severe or which is more than momentary in duration. Moreover, the society argues, there may be many forms of suffering other than pain, such as fear, hunger and illness, which should be controlled.

RSPCA's other main objections to the existing proposals concern justification and public accountability. It believes the Home Secretary should accept ultimate responsibility for the justification of licensed experiments and that he should be answerable on such questions in Parliament. However, unlike some other animal welfare groups, RSPCA wants the same strict pain controls to apply to all procedures, regardless of their purpose. The society thinks that the role and composition of the proposed new Animal Procedures Committee have not been sufficiently clearly defined, and wants greater encouragement to be given to the search for procedures that avoid the use of

animals.

The society does however welcome the restrictions on the use of animals for educational purposes and the increased scope of the licensing system. But there is a danger, the society says, of the system becoming an unworkable bureaucracy. Applications for licences should be accompanied by two separate sponsors, the second to be chosen by the Home Office from a list of acknowledged experts, and the proposed act should refer to a code of practice specifying standards of animal care and husbandry.

The Home Office is now considering various comments it has received on the white paper, and will shortly be inviting welfare groups — including RSPCA — to a meeting to discuss their views. Subject to availability of parliamentary time, a bill could be debated in about a year's time.

Tim Beardsley



Jammu, India

THESE fossil tusks are still lying where they were found a few years ago by geologists — near the village of Lovely some 40 km east of Jammu in the Swalik hills of India. The largest tusk, thought to be the biggest ever found, is approximately 4 m long and up to 25 cm in diameter.

The tusks have been identified as belonging to an extinct species, *Elephas ganesa*, the immediate ancestor of present Indian elephants. Although protected from the elements by a shed, the tusks have had no preservation work performed on them so are gradually deteriorating.

The previous "record tusk", some 3.25 m long, was also found in the Jammu area ten years ago and is now in the museum in Jammu City.

Suraj Saraf

High-technology magnets

UK company goes public

THE Oxford Instruments Group successfully went public last week. The company has attracted much interest in recent years among watchers of high technology companies. Starting from the proverbial garden shed in 1959, it now has a virtual monopoly in the United Kingdom in high performance superconducting magnets for nuclear magnetic resonance (NMR) whole-body scanners and last year, had a turnover of £26 million and made a profit of £2.7 million before tax. The company cashed in on this interest last week, when the first public share offer was oversubscribed by 6.8 times at a striking price of 285 pence per share, giving the group a paper value of more than £126 million. Shares had been offered for sale by tender, with a minimum tender price of 230 pence per share. As a result of the sale, 18 per cent of the capital will be publicly owned.

The immediate spur for last week's offer was the need for a new factory to build large magnets for body scanners. The market for such systems is expected to expand rapidly over the next few years, as NMR avoids exposure to damaging X rays used in existing scanning systems. Recent research by Professor Peter Mansfield at the University of Nottingham has also shown the potential of NMR scanners to produce moving pictures, useful for diagnosis of, for example, heart conditions.

Mr Martin Wood, the company's deputy chairman, founded Oxford to exploit research being carried out at the University of Oxford's Clarendon Laboratory, and links with university departments and other research institutions have grown. Many observers see the Oxford Group's organization as a model for successful technology transfer, divided as it is into seven semi-autonomous operations, each catering for a different product area. Products now include NMR spectroscopy and cryogenic systems for research use, ambulatory patient monitoring apparatus and diagnostic aids, as well as industrial analysis and control equipment. More recently, the group has started to manufacture plasma and ion beam milling equipment for semiconductor manufacture.

Wood has now handed over day-to-day running of the company to his collaborator of many years, Barrie Marson. Wood now plans to devote more of his time to making similar successes out of other ideas that are still hidden in laboratories. Few can be better qualified to undertake such an exercise. Oxford has had its share of problems during its 25-year history, most of them traceable to reluctance on the part of financial institutions to put large sums into an obscure-sounding idea. But with a good track record, Wood may find the going easier.

Tim Beardsley

Israeli irrigation

Making the most of little water

Rehovot

RAIN-making has come of age in Israel. Starting in October, immediately after the Feast of Tabernacles, religious Jews have traditionally prayed for rain and dew in the Holy Land. Two generations ago in Jerusalem, Sephardic Chief Rabbi Avraham Gagin led the prayers, and now his grandson, a Hebrew University meteorologist also named Avraham Gagin, is Israel's chief rainmaker.

Professor Gagin and his colleagues claim that cloud seeding has achieved an annual increase of precipitation of approximately 15 per cent. Two basic methods are used. The first is to seed clouds from light aircraft with silver iodide particles — carried in vapourized acetone. There is also a supplementary system using cannon-like generators strategically located in the mountainous northern part of the country which can discharge silver iodide particles into the sky when the appropriate clouds are present.

The ability of Israeli meteorologists to determine exactly which clouds to seed, as well as their exhaustive statistical and physical studies of the results of seeding programmes, have brought many requests for guidance. This month, for example, Professor Gagin will fly to Mexico to talk to rainmakers there. And although Israel's Arab neighbours might have objected to cloud seeding as a means of "stealing their rain", Israeli efforts have in fact increased rainfall in neighbouring Jordan.

Conservation nevertheless remains the backbone of Israeli concern for water supply, and attention is focused on agriculture since more than 80 per cent of local water still goes to farmers. Dr Gerald Stanhill of the Volcani Agricultural Research Centre says that the water delivery and irrigation systems now used in Israel ensure that four-fifths of the water allocated for irrigation actually passes through the crops under cultivation, contributing to their growth and yield. This contrasts with a world average of less than 50 per cent.

The hallmark of Israeli practice is that water reaches the fields in closed pipes instead of through canals and ditches which are prone to seepage and evaporation and because, being under pressure, it can be applied exactly where and when required. Drip irrigation, a system developed in Israel whereby water — sometimes mixed with chemical fertilizers and even pesticides — reaches individual plants through perforated plastic pipes (rather than being spread over an entire field as with other irrigation systems), also saves water.

A refinement of this system is now being tried out by Dr Israel Levin, a kibbutznik attached to the Volcani Centre, who is using plastic pipes not on the surface but

about 40 cm below ground level. The tendency for the little holes in the pipes to be clogged by the roots of the plants under cultivation has been overcome by adding a herbicide to the water, which kills the roots of the plants around the holes without affecting roots elsewhere.

Fertilizer supplied through the irrigation system (known as fertigation) is especially effective in orange groves, according to Dr Hanoch Bielora and his colleagues at the Volcani Centre. They say that fertigation can increase yields by as much as a third and, because the system is more selective, economize in fertilizer and thus minimize the danger of ground water being polluted by nitrogen.

In the spirit of making every drop of water count, another Volcani research group headed by Dr Yehezkiel Cohen is individually metering the quantity that passes through single trees or even single plants. Using heat pulses in the trunk or stem as flowmeters, they can directly

measure water requirements and so adjust the irrigation programme accordingly.

Dr Stanhill and his Volcani colleague Dr Ehud Stibbe are particularly concerned with the energy cost of Israel's sophisticated water-supply system. They point out that moving water from one place to another under pressure requires a great deal of energy and that piping water to farms under lower pressure requires less fuel.

Conservation would also be served, says Professor Dan Yaron of the Hebrew University's faculty of agriculture, if farmers were charged a more realistic price for water. Such a move might even eliminate production of certain water-intensive export crops which are now sold at a loss to the national economy. Professor Yaron points out that a decline in the demand for water would eliminate the need to develop new resources, "which is increasingly expensive because easily exploitable sources have long since been harnessed". The money saved, in Yaron's view, could be used for other development projects that might bring greater benefits to rural areas.

Nechemia Meyers

Australian technology

Search for improvement begins

Canberra

THE new Australian Government is setting great store by technology. In a speech at the opening of the national technology conference last month, the Prime Minister, Mr Bob Hawke, for the first time saw new technology as playing a central rather than a peripheral role in a strategy for economic recovery. "Industrial innovation is essential to Australia's future economic well-being, not only in industries producing glamorous new products, but throughout established industries as well." This was Mr Hawke's strongest statement yet in support of the stand taken by his Minister of Science and Technology, Mr Barry Jones, who has long been cajoling and admonishing a recalcitrant nation to sit up and take notice. The Prime Minister's remarks contrast with the view expressed by his cabinet only a few months ago, before the April economic summit, that a paper by Mr Jones was "too alarmist" to be presented on that occasion.

Mr Jones's opening speech last month, based on that ill-fated paper, was largely a reiteration of his familiar theme. "Australia has become an industrial museum and our factories are working models of the age of chisels, spanners and hammers . . . Managers and investors are often unaware of the technological and research capacity that surrounds them." He pointed out that Australia, with 0.3 per cent of the world's population, produces 2.0 per cent of its scientific papers but ends up with only 0.1 per cent of the world's high technology output and ranks twenty-second out of the 24 nations of the

Organization for Economic Cooperation and Development (OECD) in *per capita* exports of technology-intensive products.

Bringing together academics, captains of industry, trade unionists, bureaucrats and politicians, the conference organizer — the Department of Science and Technology — posed specific questions to groups of participants to establish agreement on how to draft a "national technology strategy". Consensus, however, was noticeably lacking on key issues such as the need for selective assistance to specific "sunrise" industries, or on what needs to be done about the employment-displacing effect of new technology. There was agreement only on topics over which there can be no dispute — the private sector ought to invest more in research and development, companies must revitalize themselves by adopting new technology, education is important and there should be greater interaction between research institutions.

One contribution at the conference was the suggestion that any subsidiary of an overseas company established locally should be completely autonomous with regard to investment and management decisions and the Foreign Investment Board should try to insist on this.

Unusually, this conference did not break up on a self-congratulatory note. Mr Jones made clear in his closing address that he was dissatisfied with the outcome and despaired of reaching consensus in time to make effective technology policy. "The sleepers may be waking, but they are still very drowsy" he concluded, in an allusion to his book *Sleepers, awake*. **Vimala Sarma**

Journal citations

SIR — It has been suggested in this correspondence column by P.C. Reid¹ (and elsewhere by this writer²) that it would be desirable to have a standardized system for journal citations.

The *Journal of the American Chemical Society* has recently changed its style to one which is similar to that used by the Royal Society of Chemistry. A citation in the RSC style would be as follows:

S. Kontainen *et al.*, *Nature*, 1980, 274, 477-480

This style would appear to have several advantages over the style currently adopted by *Nature*: it is surely more courteous to give initials before surnames, more logical to give the date of publication immediately after the journal title and more convenient to give the page reference last.

Any move towards a standardized system must be preceded by debate over the merits of the various rival systems. Perhaps this letter will initiate such a debate and contribute to further progress on this topic.

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2. Akeroyd, F.M. *Chem. Br.* 18, 481-482 (1982).

MR Akeroyd's call for a debate on the subject of reference citation styles has in fact been answered. For many years the supporters of rival systems — the "Vancouver style", the "Harvard" system and others — have been discussing the merits of the various alternatives. So far there seems little sign of a general agreement on a single universal system. Each journal has its own requirements. The merits of the system used by *Nature* are chiefly brevity and the absence of excess wordage (strings of authors' names and dates) from the text of a manuscript. — Editor, *Nature*.

Numerical verbs

SIR — English spelling is ridiculous. Nevertheless, the language is widely used because it has simplified grammar by discarding many unnecessary features such as gender (when unconnected with sex) and most verb endings. An interesting editorial article¹, by saying "... whether disagreement between the number (singular or plural) of a verb and what appears to be its subject is a mistake or a pointer to some hidden meaning", seems to find merit in the survival of the terminals in the third person singular of the present tense. We can write unambiguously without similar verb endings in other tenses. Would it not be sensible to abandon that residual complication officially? West Africans and West Indians have, to a large extent, already done this unofficially.

This course is followed with the verb *can* in its original sense of "to be able", but not

in the sense "to put in a container". American usage does the same with *fit*. Thus S.J. Gould writes "This view fit nicely with most nineteenth century concepts ..." (ref. 2). Many other examples from equally careful American writers could be quoted. Can anyone explain why, in North America but not in Britain, *fit* shares the sensible simplicity of *can*. Americans do not here seem to be following an older British form. Shakespeare wrote "Why dost thou laugh? it fits not with this hour". (*Titus Andronicus* Act 3, sc.i, 266). Martius's comment applies with equal force to those who consider the rationalization of English a trivial matter.

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1. *Nature* 305, 477 (1983).
2. Gould, S.J. *Ever since Darwin*, 37 (Pelican).

Similar, but not homologous

SIR — The literature of homology is beset with a confusing profligacy of terms (many reviewed by Haas¹), and Moore² does a disservice by adding another. Homology is a simple concept; it is resemblance (more precisely, correspondence) caused by a continuity of information³. It applies within individuals as well as among them, to lineages as well as their descendants, to molecules or genes or behaviours or cultural artefacts as well as to anatomical structures. Moore's distinction is based merely on how we discover homologies, a sometimes difficult problem⁴ but not one relevant to the nature of homology itself. Homology within individuals, an important developmental phenomenon, should never be used as a criterion for homology among individuals, which is due to descent from a common ancestor, despite the temptation to do so exemplified by the "premolar-analogy" theory of mammalian dentitions or the foliar theory of the flower considered phyletically³.

For molecular biologists, many of whom still ignore the evolutionary half of biology while trying to practise it, a good touchstone is that homology is always an inference, never an observation. What we observe is similarity or identity, never homology.

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2. Moore, R.T. *Nature* 305, 268 (1983).
3. Van Valen, L.M. *J. Morphol.* 173, 205-312 (1982).
4. Renate, A. *Die Grundlagen des Natürlichen Systems* 2nd edn (Geest & Portig, Leipzig, 1956).

Chaos at random

SIR — As a long-time worker in the non-linear vineyards, I was delighted recently to see that you have discovered chaos^{1,2}.

You correctly recognize that even the most evangelical worker in chaos stops short of uttering the word random to avoid the wrath of the gods of chance. At the recent Swinney-Gollub conference on classical chaos, for example (Testing Nonlinear Dynamics, 6-9 June, Haverford College, Pennsylvania), the speakers pushed their nonlinear banners as far as the daring, but still vague, Deterministic Chaos but avoided Deterministic Randomness in fear of a contradiction in terms.

We now have an opportunity to rescue nonlinear dynamics from vague, weasel words such as irregular, unpredictable, erratic and chaos¹. Rejoice, chaos and random are synonyms! The meek may continue to use the historical and safe chaos, but the daring may elect random instead. The surprise is that the shift from chaos to random is much more than name calling.

Now that we know precisely what chaos = random is, we can ask whether or not quantum mechanics has any — a question raised in *Nature* in May². It was also raised at the recent Conference on Quantum Chaos (20-25 June, Como, Italy). The prevailing sentiment (but no proof) was that finite, bounded quantum systems do not have any randomness (chaos) beyond that contained in the quantum wave function itself. Moreover, algorithmic complexity theory offers the hope of a definitive proof. One should note that the quantum systems studied by R.V. Jensen are not bounded, finite quantum systems. Randomness in infinite quantum systems remains a totally open question.

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2. *Nature* 303, 15 (1983).
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Flies scarce

SIR — In an extensive interdepartmental study designed to examine the efficiency of E.G. Gray's method for swatting flies (*Nature* 25 August, p.678), we have found out of every three flies challenged, three are killed. However, since we have only been able to find three flies since E.G. Gray's letter was published, your readers may wish to consider the possibility that flies not only use their "visual-brain-motor system" to avoid incoming swats, but also to peruse the pages of your esteemed journal for matters of dipterous interest.

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Gravitational waves waves on Sun?

The notion that a solar oscillation may be stimulated by a train of gravitational waves from a nearby binary star, however preliminary, is an intriguing pointer to novel methods.

A BOMBSHELL was hidden among the more political announcements of the director-general of the French Centre National de la Recherche Scientifique (CNRS) last week — a claim that French astrophysicists, in conjunction with Italian and British colleagues, had detected gravitational waves impinging on the Sun. The waves, the group said, are coming from the gamma and X-ray source Geminga, and can be detected in the form of a solar oscillation.

The evening newspaper *Le Monde*, given early warning of the announcement, made it a front-page lead on 11 October with the headline "A major discovery in astronomy — confirming a theory of Einstein", while earlier that day Philippe Delache, the prime mover of the group, was holding up a proof of the front page to a European Space Agency committee meeting at Frascati near Rome. The committee was deciding between various proposals for new scientific satellites, among them SOHO — one of whose missions is to measure solar oscillations. SOHO was one of the satellites ultimately selected, although this may have had nothing to do with Delache's presentation (see p.660).

But, said Delache later, "we must be very, very cautious" in the interpretation of the data. His view was echoed by the British partner in the potential discovery, George Isaak of the University of Birmingham.

The tale, so far unpublished, is this. At two scientific meetings during the summer, Isaak had raised an interesting possibility: that the Sun might be used as a detector of gravitational waves. First, its mass (which determines coupling to gravity) is obviously much larger than any experimental apparatus on Earth. And second, although this mass is concentrated at the centre (where the density is $100,000 \text{ kg m}^{-3}$) a gravitational wave which shook the centre could propagate out to the Sun's surface and be detected there as a solar oscillation. Moreover, if it propagated as a pressure wave (a sound wave), its amplitude would grow as it rose into the more rarefied layers of the Sun, amplifying by perhaps a factor of 100,000 from core to surface "like the cracking of a whip".

Then, "in a flippant mood", as Isaak describes it, he suggested that the solar 160-minute mode — normally regarded as far too long to be a pressure wave but a mode whose amplitude is difficult to explain on any basis — might in fact be driven by the absorption of a gravitational wave at

that frequency. Delache (of the Observatoire de Nice) heard the suggestion, and set to work with Jacques Paul (CEA, Saclay) and G. F. Bignami (CNR, Milan) to see if there was any sign of a 160-minute mode in the gamma radiation of Geminga, whose radiation had previously been found to be steady.

Why Geminga? Because the steepness of its X-ray spectrum — indicating little interstellar absorption — in combination with a very low visual magnitude (21.2 according to an identification by the Canada-France-Hawaii telescope) suggests that Geminga might be the closest condensed object to our Solar System. Then, if it were a binary condensed system, and if it had a frequency of 160 minutes, it could be radiating gravitational waves that could drive the Sun's 160-minute mode.

So Delache and company set out to make a fast Fourier transform of five months' gamma observations, spread over seven years, of Geminga from the satellite COS-B. And they found, they say, a 160-minute period. Taking two months' data alone, the 160-minute signal is detectable, says Delache, and it increases in amplitude as 3, 4 and 5 months' data are taken. Background radiation at the same intensity showed no 160-minute signature.

Moreover, the Geminga period does not exactly match the period of the solar oscillation — it is exactly one cycle per (Earth) year different. This would be explicable if the mode stimulated in the Sun has a non-zero azimuthal number m , or in other words, if it travels around the solar equator. For then an Earth-bound observer would see one cycle more (or less, according to the direction of travel of the wave) each year than a stationary observer. Since we do not travel around Geminga, however, we see that at its true frequency. That m of the 160-minute mode should be non-zero then becomes a testable prediction of the gravitational wave hypothesis.

So far, so good — but there are difficulties. If the 160-minute mode is not a pressure wave, Isaak's amplification argument does not apply, as he himself points out. "There is a problem with the amplitude", Delache concurs, at both ends of the radiation: at Geminga and at the Sun. The power of a binary gravitational radiation source (the radiation comes from the advance and retreat of the two masses) varies as the fifth power of the mass, says Delache, but Geminga's mass is unknown. And at the Sun, the dynamics of the

160-minute mode are not understood.

Furthermore, a British solar theorist pointed out last week that the gravitational radiation from a binary is emitted predominantly at twice the orbital frequency, whereas the gamma radiation would vary at the orbital frequency, unless our line of sight lay in the plane of the orbit (possible, but unlikely). Delache is undaunted by this argument, however: perhaps each of the condensed partners has gamma-emitting hot-spots, he suggests.

Delache does point out, however, that there is some evidence of a 160-minute period in microseismicity of the Earth which could be explained by the further influence of Geminga's gravitational radiation on the Earth. Much now seems to depend on the theorists, and the calculation of amplitudes; but meanwhile Delache and colleagues are going on to analyse the X-ray radiation from Geminga as detected by the Einstein Observatory.

More news about gravitational waves has come this week from Eduardo Amaldi and his group in Rome, who are seeking gravitational waves with the now more traditional suspended bars of metal. They were the reluctant source of Italian headlines claiming a source of gravitational radiation at the centre of the Galaxy. "We didn't want to publish anything", Amaldi said this week. Amaldi and his group have been studying background in their detector — single bar spikes — "and we've found some periodicity; but they're rather large signals and we don't believe they are gravitational waves", Amaldi said.

However, "one of the periods" is identical with the sidereal day (the true period of rotation of the Earth relative to the fixed stars), and the maximum of the cycle is "not far, within rather large errors" from the direction of the centre of the Galaxy.

And strangely enough, the very first claimant of the discovery of gravitational waves — Joe Weber of the University of Maryland — also believed his waves were coming from the galactic centre. Could it be, perhaps, that there is a galactic source, and that it is not the detector itself but the Earth that is reacting to the waves, transmitting the effect through seismicity? It is difficult to say until the work is published.

So when will Amaldi publish? "It's a long business" he said. "We are very very careful. These are very small effects." But with large consequences, one might add.

Robert Walgate

Techniques

Scanning tunnelling microscopy

from J.B. Pethica and M.D. Pashley

THE new scanning tunnelling microscope developed at IBM's Swiss laboratories by G. Binnig and co-workers¹⁻³ looks certain to provide a powerful new way of tackling one of surface sciences most important problems — the determination of surface atomic structure.

Most current techniques involve broad-beam scattering or diffraction, and include such methods as low-energy electron diffraction (LEED), low-energy atom scattering or glancing-angle X-ray diffraction. All require fairly complex and sometimes model-sensitive calculations to determine surface structure. The direct real-space surface imaging that has always been sought, particularly for weakly periodic or disordered structures, may finally have been provided by the new microscope.

The microscope operates through the positioning of a sharp tungsten tip about 10 Å above the flat conducting surface of interest. With the application of a small voltage, electrons may tunnel across the vacuum gap. The gap is held accurately constant by feedback positioning of the tip, via a piezoelectric mount, so that the tunnelling current across the gap remains constant. When the tip is scanned across the surface, the position of the tip follows height variations of the surface with sub-angstrom accuracy. Remarkably, Binnig and co-workers found that this height resolution was accompanied by a lateral resolution of the order of a few angstroms. Therefore, atomic scale topographical mapping is possible. In their early publications^{1,2} features such as individual atomic steps were mapped. Most strikingly, they have recently shown real space maps of the silicon (111) 7×7 and gold (110) 2×1 and 3×1 reconstructions³, all of which have been observed by other techniques.

The imaging of these known structures is at present the chief evidence that the tunnelling microscope is working as its authors believe. How is it that such good lateral resolution is obtained? The tungsten tips used are not especially sharp; the ends are simply ground. The microscope's inventors surmise that the tip end actually consists of many microasperities, of radius around 10 Å. Since the tunnelling current is so strongly dependent on separation, only the microasperity nearest to the surface will contribute significantly to the measured current. One microtip will only predominate over a limited area of the surface. As the tip is scanned over a large distance, tunnelling will occur at another microtip, mapping a new and unrelated area of the surface. This is what has been observed, individual mapped areas being

typically 200 Å across.

Two recent theoretical papers have presented models for the microscope operation and given simple quantitative relations for the experimental parameters. Tersoff and Hamann⁴ treat the tip as a single spherical potential well. A tip wavefunction is found assuming it to be purely s-like. The matrix element for tunnelling between states in tip and surface is then obtained, leading to an analytical expression for the tunnelling resistance. Based on a charge density calculation, the authors determine the corrugation height that should be observed for the 2×1 and 3×1 reconstructions on Au(110), finding good agreement with experiment. Reasonable values of tip radius (~9 Å) and vacuum gap (~6 Å) are found for the experimentally determined tunnel resistance of 10⁷ ohms at 10 mV applied potential.

In the same journal N. Garcia and co-workers⁵ present a conceptually less simple model where calculations are based on techniques developed for atom scattering. This allows fairly general tip shapes to be used. These authors find rather smaller values of tip radius (3.5 Å) and tip-to-surface separation (3.9 Å) are needed to match the experimentally observed corrugation heights.

These theoretical models give a simple picture of the microscope operation. More detailed theoretical models will probably need to take account of the atomic structure of the tip which may well consist in effect of one or two atoms. The tunnelling process itself may give much

further information. For example, Binnig and co-workers have demonstrated 'resonant' tunnelling oscillations; these occur at voltages high enough for electrons to field emit from one surface and 'resonate' in the remaining gap to the second surface. From the voltage dependence of these oscillations, an estimate of the tip-to-surface distance was made. Further information is likely to come from the use of different tip materials and measurement of a wider variety of surface geometric structures. The microscope is evidently at a stage of development where detailed experimental studies are required in order to characterize its mode of operation.

Tunnelling microscopy has great potential for further development. Measurement of inelastic processes in the tunnelling current would provide a very high spatial and energy resolution surface spectroscopy. This will give further information on the tunnelling process and surface electronic structure and also open up new possibilities for investigating adsorbed molecules. The microscopy will be applied to crystal and thin film growth phenomena and to imaging of disordered surfaces. Other applications and expansions upon the technique abound. □

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Accretion disks

How the wind blows

from Marek Abramowicz and Geoffrey Bath

IN a recent issue of *The Astrophysical Journal* (271, 70, 89; 1983) Begelman, McKee and Shields greatly extend earlier studies on coronae and winds above accretion disks.

Accretion disks operate through the action of viscous stresses. Gas orbiting a compact central body gradually loses its angular momentum through 'friction' to gas further out. As the balance of centrifugal force against gravity weakens in gravity's favour, the inner gas slowly spirals further inwards. Viscous stresses not only drive accretion by transporting mass inwards and angular momentum outwards, but they also convert the gravitational energy of matter into heat. The heat diffuses towards the top and bottom surface, and is radiated by the disk

photosphere. Radiation from the hot innermost region can be absorbed by much cooler outer parts and heat them up, forming a tenuous corona and blowing off a wind.

The authors of the new paper assume disks to be geometrically thin, ignore effects of radiation pressure and consider cooling and heating of the corona and wind by Compton scattering only. Quite apart from giving a detailed mathematical model of Compton-heated coronae and winds, by itself of significant astrophysical importance, they make a surprising theoretical discovery — the mass loss rate of the wind may be greater than the rate of accretion flow through the disk. Obviously such dramatic mass loss (accompanied by substantial loss of angular momentum) may

qualitatively change the standard picture of conservative accretion built up by theoreticians in the past ten years. This could be crucial for understanding all those astronomical objects which are believed to contain accretion disks: X-ray binaries, active galactic nuclei and cataclysmic variables. But is there observational support for the idea of strong winds?

Recent observations of partial X-ray eclipses in two X-ray binaries with low X-ray luminosity indicate the existence of very extended X-ray sources and have been interpreted as X-ray scattering by a hot corona or wind above the accretion disk. Although the existence of coronae and winds is almost certain, no direct observational clues to the presence of very strong winds have yet been found. Large fluctuations of luminosity observed in some of the X-ray sources may be, as Begelman, McKee and Shields point out, connected with some form of unstable bimodal behaviour driven by strong winds. However, as many alternative explanations are possible, this evidence is by no means conclusive. Since polarization properties of X-ray radiation depends strongly on the geometry of winds and outer coronae, polarimetry measurements may provide a future test for the reality of Compton-heated, strong winds.

In active galactic nuclei (quasars and Seyfert galaxies) it is widely held that the accretion structure is strongly influenced by radiation pressure. The predicted shape resembles a very fat torus rather than a thin disk. This contradicts the assumptions used by Begelman, McKee and Shields, but should not be taken to mean that a corona and wind cannot exist above such a thick torus. On the contrary, coronae and winds are probably the places where the non-thermal part of the active galactic spectrum originates. Instabilities driven by a strong wind do not depend crucially on any particular disk geometry or structure, so one part of the model of Begelman, McKee and Shields can still be applied. Although observed variations of luminosity seem to agree with the strong wind hypothesis, they can be explained in many different ways. Strong winds could solve the problem of how to generate and support the clouds that are believed to be necessary to explain the emission lines in quasars and Seyfert galaxies. Other problems arise, however.

The efficiency of accretion can be defined as the number of ergs of energy radiated per gramme of matter processed by the disk. With a strong wind present, more mass must be supplied to the system to maintain the same luminosity. This means that the presence of a wind decreases the efficiency of accretion: when strong winds blow, the efficiency is low. Observations, on the other hand, seem to indicate a rather high efficiency.

With no obvious observational support for the idea of strong winds let us turn to the theoretical side of the story. The

strength of the gravitational field increases close to the centre. Thus, the gravitational energy released by a lump of gas falling down from the outer parts of the disk (the base of the wind) to the centre is greater than the energy needed to blow the same lump upwards from the wind base to infinity. This makes it possible, in principle, to power an expelled mass flux with intensity greater than the downward flux of matter. If there were an efficient mechanism for transporting energy from the central to the outer parts of the disk, then strong winds will blow. For this to occur, however, strict conditions on both the vertical structure of the disk and the opacity of the wind must be fulfilled. Discovering whether these conditions are met by real disks is far from straightforward. Theoretical models of the vertical structure of accretion disks are highly uncertain, primarily because of the dependence of structure on the unknown nature of viscosity. Normal stellar winds are much simpler and much better probed by observation than those discussed in the paper of Begelman, McKee and Shields. Yet even in this simple case astronomers cannot agree on how to compute the intensity of the wind. Some experts argue that,

because the wind is not a thermodynamically closed system, its intensity cannot be worked out in a simple way from calculations of thermal balance between heating and cooling but must be treated as a free, phenomenological parameter.

The theory of Begelman, McKee and Shields gives the best model of the Compton-heated coronae and winds which is available today. It makes the concrete suggestion that extremely strong winds are possible. This could be crucial (if it is true) to future understanding of accretion disks. The uncertainties, both of a theoretical and observational nature, only underline the astrophysical significance of the theory of windy disks. 'The essence of a good mathematical model,' said Karl Popper, 'is that it should embody the bold ideas, unjustified assumptions and speculations which are our only means of interpreting nature. The good scientist then puts his model to the hazard of refutation.' □

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Pathology

What origin for toxic shock syndrome?

from Richard W. Lacey

THE hypothesis, advanced earlier this year¹ that toxic shock syndrome is the result of toxins produced when the familiar bacterium *Staphylococcus aureus* acquires a new bacterial virus seems, after all, unlikely to be true. New work² reported in this issue of *Nature* (see p.709) shows that the genes responsible for toxic shock syndrome are not closely associated with the presence of a virus (prophage) integrated into the staphylococcus genome.

Toxic shock syndrome is of great interest because it appears to be a 'new' disease caused by an 'old' microorganism. The disease probably results from the production of a potent exotoxin by the bacterium. It causes rash, reduced blood pressure and collapse, and has harmful effects on the lungs, heart and gastrointestinal tract.

As the pathogenesis of the disease resembles that of diphtheria, where toxin is produced when a virus is carried by the bacterium, it was reasonable to suppose that toxic shock syndrome appeared when staphylococcus acquired a new prophage. The hypothesis was in accord with the good epidemiological evidence for the spread of prophage among staphylococci in nature, and was strongly supported in the work published by Steven Schutzer and colleagues¹. They were able to show that several strains of staphylococcus responsible for toxic shock syndrome did indeed

possess prophages with characteristic plating properties. The new work, however, leads, in the authors words, to the conclusion that the hypothesis that the 'ability to cause toxic shock syndrome is prophage-determined' is 'untenable'.

In most conditions, in man and in many animals and birds, strains of *S. aureus* behave as true commensals, and are found in the human nose, perianal region and on moist skin elsewhere. Disorders of the host, for example, surface wounds and diseases of the skin, permit entrance of the bacterium to the body where, after multiplication, its extracellular products, notably enzymes and toxins, cause disease. Some of these products are almost always synthesized by the bacterium *in vitro*, for example, coagulase and DNase. The presence of these are now used to identify the organism. Others, such as epidermolysin, are produced occasionally. This enzyme causes the 'scaled skin syndrome' in which the outer epidermis of neonates is separated from the basal layer of the skin, revealing the bright red colour of the deeper epidermis. The disease can be fatal, but is fortunately rare. It is due to a few related types of *S. aureus*, and although the genetic determinant may be plasmid-determined, there is little evidence that the plasmid has spread between strains in nature.

Toxic shock syndrome gained prominence because some apparently healthy women in the US using a type of tampon ('Rely', Procter and Gamble) developed the syndrome and some died. The organism was isolated from the vagina and tampon but not elsewhere. The reason for the association is still not clear, but following the removal of these tampons from the market, the incidence of toxic shock syndrome seems to have declined. The syndrome can, however, occur in women not using tampons and even in men and children. The disease is rare: estimates of frequency must consider the strength of the diagnostic criteria used, but probably less than 1,000 cases have been recorded. Most of the victims were previously healthy and the staphylococcus colonized several sites. The staphylococci incriminated are similar in many properties and probably represent the spread of a single clone — with, of course, some attendant variation. There is no reason to suppose that the toxin(s) are arising *de novo* in the cell of many staphylococci at high frequency nor is there evidence for plasmid inheritance, or transfer of the toxic shock syndrome gene(s) between cultures.

In this issue, Kreiswirth and colleagues identify the gene(s) coding the toxin as chromosomal, and of all the three modes of gene transfer that can occur in *S. aureus in vitro* — transduction, transformation and conjugation — chromosomal genes typically transfer between cultures at low frequencies. Thus, as with the scalded skin syndrome, spread of the determinant for the toxin among the general staphylococcal population is unlikely to have occurred. This is reassuring. Toxic shock syndrome is therefore best viewed as an unfortunate accident in which an extracellular product of the organism has evolved and causes ill effects in the host. There would appear to be no selection pressure favouring such an event. Cloning the toxin gene will facilitate the production of a vaccine but there will be problems of defining which patients should receive it, particularly as the incidence of the disease may already be declining.

Toxic shock syndrome is, however, an important disease and well worth further study. *S. aureus* is the commonest cause of wound infections throughout the world, and probably only eclipsed by *Escherichia coli* as the most frequent bacterial pathogen in the western world. Since the highly virulent strains of *S. aureus* that were endemic in many hospitals in the 1950s and 1960s petered out, the organism has not received the attention it deserves — as is illustrated by the spread of a multi-resistant strain throughout most of the public hospitals in Australia in the past few years. In the future, we may expect the staphylococcus to produce novel diseases, since not only may the human host alter in vulnerability, but the capacity for gene reassortment in this species is remarkable.

As early as the 1960s work by Parker and colleagues in the UK and Rosendal and

Bülöw in Denmark showed that the properties of *Staphylococcus* could be easily altered by the acquisition of novel prophages in nature. Bacteriophage typing in this organism is, in fact, largely determined by prophage carriage. Apart from the presence of restriction endonucleases in some group II strains, resistance to bacteriophage lysis is governed by lysogenic immunity and bacteriophage typing is, therefore, in effect monitoring the occurrence of alteration of prophage carriage in nature. A number of extracellular products, for example, lipase, fibrinolysin and haemolysin, have been shown to be altered by such lysogenic conversion. Furthermore, resistance to the antibiotic, gentamicin, can be due to the possession of a prophage. Resistance to other antibiotics is however, usually plasmid-determined, and

a number of other products are also encoded by plasmids. Novick and co-workers now add to the possibilities for genetic reassortment by demonstrating transposition of genes between different replicons *in vitro*. This, coupled with the ease with which plasmids transfer can occur between cultures *in vitro*, makes rapid evolution of the organism with the initiation of novel diseases likely to occur. There is no reason then to consider that the appearance of a new product, for example the toxins causing shock syndrome, is due to anything other than chance. □

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Electronics

Semiconductor superlattices

from D.A. Anderson and N. Apsley

FOR some years scientists have been making increasing use of heterojunctions to extend the performance of semiconductor devices. A heterojunction is formed when a crystal of one material is grown epitaxially on a crystal of a different material with the same lattice constant. Typical examples include double heterojunction (DH) lasers, now widely used in fibre optic communications, and heterojunction bipolar transistors intended for use at high-microwave frequencies. The control of the crystal growth techniques (molecular beam epitaxy and metal-organic chemical vapour deposition) used in making such heterojunction devices has now reached the point where alternate layers of two materials can be grown, with each layer as little as 10 Å thick. The electronic properties of such structures are very different from those of the constituents and their study is the subject of much current interest from both pure and applied scientists. Recently the Rank Prize Funds held a mini-symposium on this topic with optical and optoelectronic effects as the theme*.

The electronic properties of a semiconductor crystal are primarily determined by the form of the uppermost valance band and lowest conduction band. These in turn are governed by the atomic properties, crystal symmetry and lattice constants. Most technologically interesting semiconductors are cubic with lattice constants of order 5 Å. Therefore the ability to grow periodic multilayer structures or superlattices on a comparable scale offers the possibility of directly tailoring the supercrystal's band structure. This has been

exemplified by recent work presented by R. Burnham (Xerox Research Laboratory, Palo Alto) on GaAs multiquantum well lasers. The laser structures consist of thin (~60 Å) layers of GaAs interleaved between layers of the wider-band gap semiconductor GaAlAs. The ground-state energies for electrons and holes are increased as the well thickness is reduced, as dictated by the uncertainty principle. In this way the effective band gap of the GaAs can be controllably increased, enabling powerful solid-state lasers to be built with output photon energies tuneable between 1.4 eV, the band gap of GaAs, and 1.7 eV (visible red). The same principle, but with two different semiconductors, could be used to design lasers and detectors which exactly match the optimum wavelength for transmission of high-speed optical information through low loss fibres.

Another advantage of the control of the crystal growth emerges in devices using local 'built-in' electric fields inside the crystal. In homojunction devices control of the local field is achieved to a limited degree by designing a specific doping profile. In a superlattice, however, the ability to step the band gap or to grade it continuously on scales comparable to the energy relaxation distances, offers an additional and extremely powerful degree of flexibility. In particular, the structure may be designed such that the fields experienced by electrons and holes are quite different. For example, conventional 3–5 p-n avalanche photodetectors (APDs) exhibit excess noise because both electrons and holes contribute equally to the avalanche process. F. Capasso (Bell Laboratories) has designed a multilayer detector consisting of alternate layers of GaAs and GaAlAs in which the electrons encounter a larger step down in potential than the holes

*A three-day symposium of 30 invited scientists was organized by the Rank Prize Funds to discuss the physics and optoelectronic applications of semiconductor superlattices. The participants included 10 invited speakers from the US, Europe and the UK, the remainder being junior researchers who gave short presentations of their work in this field.

as they cross the heterojunction from GaAlAs into GaAs. The holes thus cannot gain sufficient energy to contribute to the avalanche and noise is reduced. The work has led to a concept of an all-solid-state photomultiplier using both band-gap stepping and grading to produce a sawtooth variation in the band gap.

Most of the applications for superlattices so far have been in optical devices but there is also considerable interest in other effects. L. Esaki and his co-workers at IBM are studying a superlattice which will display either semiconducting or semi-metallic behaviour depending on the bias applied to it. They are also looking into the possibilities of superlattices consisting of three materials in periodic or aperiodic sequences. K. Hess (University of Illinois) has shown that the transfer of charge from one well to a neighbouring one could provide a very fast switching mechanism, while other workers are making use of the larger exciton binding energy in thin GaAs layers to construct an all-optical bistable switch operating at room temperature. These early results are encouraging but the full potential of superlattices and the power of the crystal growth techniques that have been developed are only beginning to be appreciated.

Before this potential can be fully exploited there is still much of the fundamental physics to be explored and many aspects of the material and technology to be properly understood. Gaining access to structures of such small dimensions with physical probes is a challenge which was discussed by several of the speakers at the symposium. Of particular interest was the use of ultra-violet photoemission, with radiation provided by a synchrotron source (R. Bauer, Xerox Research Laboratories), to measure the electronic energy levels of the top one or two monolayers of the structure as it is growing in the MBE reactor. Similar experiments are being carried out at the UK synchrotron at Daresbury. A novel application of ellipsometry can be used to infer details of the degree of intermixing of components at a heterojunction interface (J. Theeton, Laboratoire d'électronique et de physique appliqué, Limeil-Brevannes) and there is evidence that the phonon structure can be radically modified in a superlattice (R. Nicholas, University of Oxford). There is controversy over whether the constituents of a superlattice need to be lattice matched. It had been understood that lattice mismatch resulted in high densities of interface states at the heterojunction but recent results obtained by G. Osbourn and his colleagues (Sandia Corporation) suggest that some degree of lattice mismatch can be tolerated and that the resulting strain can be used to add to the degree of control over the material properties. □

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Astronomy

Inside the giant planets

from David W. Hughes

OUR knowledge of the interiors of the giant planets depends on many factors. Observational data are obviously important. The Pioneer and Voyager missions to Jupiter and Saturn helped a great deal and the visits to Uranus and Neptune coming up soon will provide another big boost. New laboratory investigations of the equations of state and phase diagrams of elements such as hydrogen and helium at a range of suitably high temperatures and pressures are also proving valuable. Coupled with this, theoreticians are trying to unravel the physics of convection and magnetic-field generation in planetary interiors and cosmogonists are reconsidering the accretion mechanisms responsible for the formation of the planets at the dawn of the Solar System.

The end result is a plethora of proposed models which have a range of characteristics. Fortunately, the diversity of the predictions seems to decrease as time progresses. Also, fortunately, the interiors of the giant planets are less complicated than the interiors of the terrestrial planets. At giant-planet temperatures most molecules are dissociated; and their constituents are not solid but fluid, so conditions reach equilibrium more quickly.

The proposed models should try to satisfy the cosmogonists' present-day hypotheses as to the formation mechanism and the chemical constituents of the outer planets. The thermodynamical properties of the constituents must obviously be obeyed and the end product must fit in with the observed values of planetary density, radius, spin period, oblateness and gravitational field coefficients. Complications such as the presence of magnetic fields and the rate of heat emission from the planetary interior have to be taken into consideration. This ties in with more detailed investigations of the electrical conductivity, coefficients of heat transfer and solubility of mixtures of hydrogen and helium. Three recent findings are that all outer planets have a rocky core of about 10 Earth masses, that hydrogen transfers from the molecular to the metallic state at a pressure of about 2 Mbar and that in both the molecular and metallic regions of planets hydrogen and helium are not completely mixed.

Recent advances in the theoretical interpretation of planetary interiors are reviewed by R. Smoluchowski in *The Moon and the Planets* (28, 137; 1983).

Jupiter is assumed to have a ratio of hydrogen to helium similar to that of the Sun. Calculations indicate that this planet has a solid core of about 14 Earth masses with a density of about 22 g cm⁻³. Jupiter's total heat content is about 10⁴² erg and it is

losing this energy at the rate of about 4 × 10²⁴ erg s⁻¹. Hydrogen and helium are well mixed and the interior has a region of high electrical conductivity in which the magnetic-field-generating dynamo operates, this having an outer boundary about 0.89 jovian radii from the centre.

The Saturn model has a core of 16.5 Earth masses and radius 0.14 R_S. This is surrounded by an icy layer extending up to 0.24 R_S over which lies the metallic hydrogen regions extending to 0.59 R_S. The planetary interior contains a 'miscibility gap' which separates helium-rich from helium-poor metallic hydrogen. The progressive migration of this region generates heat which is additional to that derived from the loss of the primordial thermal energy. A differentiated metallic layer model as proposed above would suppress convection and thus account for Saturn's unexpectedly low magnetic field.

Without spacecraft data the models for the interiors of Uranus and Neptune are much more speculative. As far as size and mass are concerned the two planets are reasonably similar. A three-layer model is proposed in which each layer occupies about one-third of the radius. The core consists of 38 per cent SiO₂, 25 per cent MgO, 25 per cent FeS and 12 per cent FeO. This is surrounded by an icy layer made up of 57 per cent H₂O, 32 per cent CH₄ and 11 per cent NH₃. The top layer is an H-He atmosphere. The composition is essentially solar apart from the significant depletion of gaseous volatiles. Recent observations of the precession of the uranian ring has helped to quantify the gravitational quadrupole moment J₂. The uncertainty in the spin period is however still a problem. The quoted values for Uranus lie between 13 and 24 h, and for Neptune between 11 and 20 h. Uranus is unique among the outer planets in having no internal heat emission. This seems to be due to the insolation suppressing the thermal gradient. Even given the normal physical and chemical constraints Smoluchowski quotes eight models for the interior of Uranus and five for that of Neptune.

There is considerable debate as to the existence of magnetic fields on Uranus and Neptune. S. Durrance and H. Moos (*Nature* 299, 428; 1982) observed Lyman-alpha radiation from Uranus and concluded that this was produced by polar aurorae on that planet. A magnetic field was therefore postulated. Neptune was thought to have a more solid core and no magnetic field. R. Smoluchowski and M. Torbell (*Icarus* 48, 146; 1981) considered the properties of the icy mantles of the two planets and came to the opposite conclusion.

Clearly more data are needed and the results from the Voyager magnetometers are eagerly awaited. Even so, considering the range of opinions that persist about Earth, the collection of a little more data is not going to lead to an immediate consen-

sus about the interiors of any of the outer planets. □

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Astronomy

Planetary nebulae and stellar evolution

from Stephen P. Maran

THE central question about the central stars, or nuclei, of planetary nebulae is 'What are their masses?', for in most theories the mass is a fundamental quantity that determines the path and speed of stellar evolution. Now, a new survey, just published in the *Astrophysical Journal*¹ by James Kaler of the University of Illinois, adds fresh data from 57 large planetary nebulae and goes a long way towards both providing an answer and testing related theoretical predictions.

Planetary nebulae are thought to be the expelled atmospheres of stars that evolved beyond the red giant stage and through the asymptotic giant phase in which nucleosynthesis produces fresh supplies of several elements heavier than helium and in which convection and thermal pulses may conspire to bring these fruits of nuclear burning into the atmosphere. Thus, analysis of the chemical composition of nebulae (more easily done, in practice, than analysis of asymptotic giant-star atmospheres) provides important potential checks on evolutionary theory, while observation of planetary nuclei provides corresponding tests of the predictions of the theory for the properties of remnant cores. Most theorists would expect a range of slightly more than a factor of two in the masses of planetary nuclei.

Of special interest is Iben's and Renzini's² prediction that the chemical composition of the nebulae should correlate with the masses of their respective planetary nuclei. However, according to Schoenberner and Weidemann³ who have a different evolutionary view, the vast majority of observed planetary nuclei have masses in the limited range from $0.55 M_{\odot}$ to $0.64 M_{\odot}$. Indeed, the distribution even has a narrow peak near $0.58 M_{\odot}$. This suggestion is especially interesting because, as Schoenberner and Weidemann point out, there is an evolutionary scenario that appears capable of producing planetary nuclei with standard masses of around $0.6 M_{\odot}$ from precursor stars with a significant range in mass.

Kaler's new report¹ appears to be just what the doctors Iben and Renzini ordered. By making extensive photometric measurements of 57 nebulae at the Prairie Observatory in Illinois and combining these results with a plethora of data accumulated by other observers of these and

27 other nebulae since 1918, Kaler has been able to assemble a statistically impressive body of evidence that supports a range of about $0.55 M_{\odot}$ to $1.0 M_{\odot}$ for observed planetary nuclei. The technique used is to plot derived planetary nuclei luminosities (L) and temperatures (T) in the $\log L$ - $\log T$ plane along with the evolutionary tracks corresponding to different planetary nuclei masses. The rub, of course, is in deriving L ; to calculate luminosities, one must know the distances, but the distances, as is commonly true in astronomy, are very poorly known. The usual method of getting around this, which Kaler employs, is to assume that the mass of ionized matter in optically thin planetary nebulae is a standard value. Optically thin planetary nebulae are then standard candles and distances can be determined from nebular photometry. Unfortunately, individual variations from the standard mass may well be large (one is tempted to remark that there is no standard mass) and with this and other uncertainties in mind, Kaler states that it is not even worth assigning errors to the distance determinations for individual nebulae.

These caveats aside, the Kaler survey does show roughly the mass distribution of planetary nuclei that many theorists would anticipate, and it also reveals that nitrogen-to-oxygen abundance ratios in the planetary nebulae correlate with planetary nuclei mass, the higher values of nebular N/O being associated with higher planetary nuclei masses. Since the Iben-Renzini theory predicts that stars of high initial mass produce more massive remnant cores (planetary nuclei) than precursor stars of low initial mass and further predicts that the higher-mass precursors develop higher N/O ratios in their atmospheres (which become the planetary nebulae) through nucleosynthesis and convective dredgeup, the correlation must be very comforting to those authors, even though Kaler's newly derived planetary nuclei masses are somewhat smaller than expected for given N/O values.

It may be too soon to lean back and look for the next problem, however, for another recent survey finds at least one contrary result. Heap⁴ has extracted from the archive of International Ultraviolet Explorer (IUE) observations a considerable number

of measurements of planetary nebulae and their central stars that have been made since the January 1978 launch of the satellite. These observations have the virtue that they were made in a uniform manner with a single well calibrated and photometrically stable instrument located above the atmosphere. Moreover, the planetary nuclei, being hotter than the surrounding planetary nebulae, stand out better in the ultraviolet than in the visual and can be more accurately measured. There are about 60 planetary nuclei in the IUE sample, including quite a few that are located in optically thick nebulae of a type excluded from Kaler's survey (which emphasized large low-density planetaries) and, indeed, from the Schoenberner and Weidemann data analysis. The data set thus is more representative of the variety of planetary nebulae. Heap follows Schoenberner and Weidemann in deriving masses by plotting data together with evolutionary tracks for planetary nuclei of various masses on a plane where absolute stellar magnitude and the logarithm of R the nebular radius, are the two axes, but adds the twist that ultraviolet magnitudes are used.

The distance must be known in order to derive all these quantities and hence standard nebula assumptions similar to those made by Kaler must be made (in fact, separate standards must be adopted for optically thick and optically thin nebulae). Furthermore, the method assumes a standard expansion velocity although there is certainly a non-negligible range in observed expansion rates of planetary nuclei⁵. Thus, the data are more uniform and, overall, probably more accurately determined than those collected by Kaler, but the method of analysis requires additional assumptions. The analysis shows general agreement with the Schoenberner-Weidemann interpretation of the planetary nuclei mass distribution for thin nebulae, in contrast to the Kaler survey, but does suggest that the planetary nuclei of optically thick nebulae represent a more massive population. Heap also finds that planetary nuclei located in the galactic disc tend to be more massive than those in the galactic halo. Since massive stars are generally confined much more closely to the disc than lighter stars, this finding is consistent with the expectation that the more massive planetary nuclei are produced from the more massive precursor stars.

Regardless of which theory (Iben-Renzini or Schoenberner-Weidemann), which data set (Prairie photometry or IUE), and which analysis technique (evolutionary tracks in the $\log L$ - $\log T$ plane or tracks in the absolute magnitude- $\log R$ plane) one prefers, it seems beyond doubt that planetary nuclei evolve into white dwarfs, and astrometric solutions for three nearby white dwarfs in visual binary systems yield well determined masses of $0.94 M_{\odot}$, $0.65 M_{\odot}$ and $0.43 M_{\odot}$ (ref. 6). This sample is small, but its selection

depends on proximity to the Sun, which is unlikely to favour atypical stars, and the derived masses extend over a comfortably large range. On the other hand, perhaps it is possible to reach the white-dwarf state without passing through the planetary-nebular phase; in that case not every white dwarf has been a planetary nucleus. The nucleus of planetary nebula A63 has been found to be a member of an eclipsing binary system, leading to an estimated mass of $0.9 M_{\odot}$ (ref. 7). The planetary nucleus in A46, also a binary star, has a mass perhaps as large as $1.1 M_{\odot}$ (ref. 8). But these two objects may not represent firm evidence for high mass in planetary nuclei because in each of the binaries, only the ratio of stellar masses is well determined⁹.

Three very luminous planetary nuclei in the Magellanic Clouds, whose distances and luminosities should be beyond doubt, have masses of 0.9 – $1.2 M_{\odot}$ derived by comparison of the stellar parameters with evolutionary tracks in the $\log L$ – $\log T$ plane¹⁰. These masses cannot be much on the high side because the stellar luminosities would exceed the Eddington luminosities (at which radiation pressure disperses a star) for stars with masses smaller than those derived. Considering the white dwarf Sirius B with astrometrically determined mass of $0.94 M_{\odot}$ the planetary nuclei in eclipsing binary systems and the planetary nuclei in the Clouds, one suspects that there may be more high-mass planetary nuclei than the Schoenberner and Weidemann scheme can comfortably confront, despite the uncertainties mentioned above. Are the three Magellanic Cloud planetary nuclei atypical? Recent IUE observations of several additional nebulae in the Clouds may help to say. In any case the measurement of luminosities and temperatures for a substantial sample of planetary nuclei in the Magellanic Clouds, a project planned for the faint object spectrograph on the Space Telescope¹¹, should put the matter beyond doubt. □

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Chemistry

A novel solar cell

from Nathan S. Lewis

SEMICONDUCTOR-LIQUID junctions offer a possible alternative to conventional solid state photovoltaic solar energy conversion devices. Unfortunately, few of the semiconductor-liquid solar cell systems explored to date simultaneously meet the necessary criteria of excellent stability and high efficiency; thus, the search for novel electrode materials is proceeding with fervour. In the last issue of *Nature*, Menezes, Lewerenz, and Bachmann (*Nature* **305**, 615; 1983) reported studies of a new semiconductor-liquid junction solar cell that demonstrates excellent stability in an atmospheric environment, and simultaneously displays solar-to-electrical energy conversion efficiencies of 9.5 per cent.

The basic operating principles of semiconductor-liquid junction solar cells resemble those of solid state cells (see *News and Views* **733**, 687, 1982). When a semiconductor is immersed in a liquid electrolyte with a suitable oxidation-reduction species present, a junction is spontaneously formed. This junction allows efficient photovoltaic action to occur, and provides a mechanism to harness incident photon energy into useful electrical work.

In addition to accomplishing the desired chemical reactions, the photogenerated carriers can also undergo side reactions which lead to decomposition or passivation of the semiconductor itself. This is a general phenomenon for photoanodes in aqueous solution and severely limits the lifetime of semiconductor-liquid junction devices. Finding a way of minimizing decomposition reactions while maintaining high photovoltaic efficiencies is crucial to the development of useful interfaces. Approaches include the use of nonaqueous solvents to minimize decomposition, and the addition of reagents to aqueous electrolytes which might compete favourably with the decomposition pathways.

Menezes and colleagues find that a n - CuInSe_2 anode immersed in an aqueous iodide solution exhibits negligible photocorrosion processes and 9.5 per solar efficiency. Significantly, the iodide-iodine solution does not have to be stringently sealed from the atmosphere in order to maintain efficient operation. The authors also point out that a simple chemical reaction of the interface can be used to promote cell stability. In the case of the CuInSe_2 device, *in situ* film formation induced by the addition of Cu ions to the electrolyte solution yields anodes which are stable for periods of over five months. The system thus compares favourably with the best stability observed in any semiconductor-liquid junction, and signals that it may indeed be possible to find specific

semiconductor-liquid combinations that will be durable over the long periods necessary for successful terrestrial photovoltaic operation.

An important point raised by Menezes and colleagues is that the CuInSe_2 semiconductor material used in their cell might be advantageously employed in a thin film electrode configuration. This suggestion is made because the absorption edge of the CuInSe_2 is of the direct bandgap type. The optical absorption coefficient of the semiconductor at a particular wavelength of light determines the profile of carrier creation, with stronger absorption implying carrier creation closer to the junction. In general, there are two distinct sources of photovoltaic action: diffusion currents and drift currents. Drift currents arise when the photogenerated carriers are created within the region of the junction field, while diffusion currents result from deeply penetrating photons which create carriers in the bulk of the semiconductor. Since the diffusion currents are substantially more sensitive to impurities in the crystal than are the drift currents, the strong optical absorption by the CuInSe_2 allows use of thinner, and less perfect samples than would be required of semiconductors such as silicon. This advantage, if exploited, could enable fabrication of photovoltaic cells in a thin film assembly without substantial loss of efficiency. However, the authors have so far restricted their investigations to the behaviour of the more expensive single crystal CuInSe_2 , and a judgement on the behavior of polycrystalline materials must await further study.

At present, the relative merits of semiconductor-liquid junction cells versus conventional space cells is a matter of debate; indeed, there is controversy over estimates of the economic feasibility of any large scale terrestrial solar device. With regard to an actual energy conversion application, it is clear that the technology of semiconductor-liquid junction cells substantially lags behind that of solid state systems. It is equally clear, however, that progress in the area is rapid, and that the basic science of the semiconductor-liquid interface poses a challenging scientific problem. Investigations which advance our understanding of the chemistry at any semiconductor junction, including liquid interfaces with CuInSe_2 , serve to provide a valuable piece of the scientific puzzle involved in the development of alternative energy sources. □

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The Earth and its mountains

SIR — I would like to comment on the diastrophic assessment of the relative merits of the three contending theories of mountain-building¹. In my 1976² and 1978³ papers, I pointed out that in relating the apparent secular accelerations of the Moon and Sun to the lunar and solar couples N and N' , the correct equation for the rate of change of angular momentum of the Earth is $(d/dt)(C\omega) = -N - N'$, whereas in all six editions of his book *The Earth*, Sir Harold Jeffreys has adopted the form $C(d\omega/dt) = -N - N'$, which tacitly assumes the moment of inertia C to be unchanging with time. This leads to quite unacceptable results if followed out straightforwardly, whereas Jeffreys handled the treatment in a way that obscures that the conclusions claimed do not in fact follow. Copies of these papers were duly sent to Sir Harold, but his only comment was, "Your references are out of date", which they were not, whence my 1980 paper⁴. There can be no question of our being at loggerheads over it, for the day is long since past when there could be any dispute as to whether a dynamical equation is correct or not.

In his recent article⁵, Jeffreys states "that whether the Earth was hot or cold (initially) does not matter much for its history". But this is quite wrong, for if it started cool enough to be solid throughout, the known seismic data⁶ for the mantle prove that the initial radius would have been 370 km greater than the present radius of 6,371 km. This is more than ten times the amount that thermal contraction could provide even when every factor is taken to maximize the latter. It implies a total reduction in surface area of about 50×10^9 km², and a redispersion of more than 150×10^9 km³ of rock, as is shown in my recent book⁷. This would be quite adequate for the more than 20 major periods of mountain-building literally unearthed by the geologists. Yet in a review article⁸ Jeffreys writes of the thermal-contraction hypothesis, "but no other suggested hypothesis even begins to explain it" (the needed contraction).

This hardly looks as if he "meant to refer to my 1965 paper"⁶ omitted from both the 1970 and 1976 editions of *The Earth*, as he now says was his intention, but Jeffreys also omits any reference to my 1972 paper⁹ wherein it is shown how all the terrestrial planets must have been formed initially solid throughout. The Moon and Mercury can hold no atmosphere now, and Mars barely so, and thus can never have passed through a gaseous or molten stage. Thermal contraction is therefore ruled out as an admissible hypothesis, and besides, where are the folded and thrust mountains on Mars, Mercury and the Moon? (Volcanic mountains are not yet involved.) That there would be no such mountains on these bodies was predicted by the phase-change theory before any of the Mariner flights,

and also that there would be no measurable dipole magnetic field on Mars, since the central pressure is far too low to produce the phase change.

On the identification of the core material with iron, the seismic data show that its uncompressed density (without change of state) would be only about 6 g cm⁻³, whereas iron and nickel, even when in liquid form, have densities 15 per cent and 30 per cent greater than this figure. Shock-wave experiments have also been adduced to show that the core is of iron, but by arguments that are thermodynamically quite unsound. In the seismic case, infinitesimal waves and pressure differences are propagated through material already under high pressure and fairly high temperature, whereas with shock waves a severe impulsive shock moves into unstressed material at a normal temperature. The treatment history to be accorded to a volume element of material is totally different in the two cases, and chemical identification by comparison of velocity-density curves, which in fact fit very poorly if at all, is not possible. The alleged agreement with iron is rendered further suspect by the feature that, if the method were valid, the arguments would lead much more strongly to the conclusion that the mantle is made of aluminium. All this is explained in detail in another relevant but unreferenced paper¹⁰.

As for the vast verbal and pictorial literature of plate tectonics with its large number of purely asseverated assumptions, it may surprise some to learn that it simply fails to qualify as a scientific theory. I am sure Jeffreys fully agrees with this. Long ago, the great Poincaré explained that "such descriptive accounts are not the role of physical theories, which should not introduce as many or more arbitrary constants (or verbal assumptions) as there are phenomena to be accounted for; they should establish connections between different experimental facts, and above all they should enable predictions to be made".

The real problem of orogeny is to account for over 20 major periods of mountain-building, and not just the one that produced the existing systems of folded and thrust mountains. This the phase-change theory is able to do, as the mere calculation of the initial radius of an all-solid Earth alone makes abundantly clear.

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Numerology puzzle

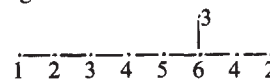
SIR — Although numerology may be tempting, it does have some place in scientific work. An illuminating example arises in the theory of finite simple groups. The largest finite simple sporadic group has order approximately 10^4 and is known as the Fisher-Griess "monster". Its smallest complex faithful linear representation has dimension 196,883.

Two remarkable pieces of numerology stem from this group. First, the coefficient q in the Fourier expansion

$$j(z) = q^4 + 744 + 196884q + \dots$$

$$(q = \exp(2\pi iz))$$

of the classical elliptic modular function satisfies $196,884 = 196,883 + 1$ (see ref. 2) and second, the group is generated by a class of elements of order two (the Fischer involutions) such that the product of any two lies in one of nine conjugacy classes with the orders of the products given by the coefficients of the highest root as indicated in the accompanying E_8 extended Dynkin diagram.



Neither of these coincidences is understood, although it has been shown that each conjugacy class of the "monster" is parametrized by a modular function whose coefficient of q^n ($n \neq 0$) is the character value afforded by a representation H_n on an element in the class.

The numerological observations provide a numerical link between two initially unrelated areas: the recently developed theory of finite simple groups and, in the first case, the classical theory of modular functions, namely Lie theory, Kac-Moody theory and singularities. It is this critical contextual information which needs sifting if a coherent explanation for the numerology is to be found.

In the example cited in ref. 1, we are presented with powers of π but no suggested context. If one believes the evidence is strong enough to demand an explanation (and this is a matter of subjective probability), then one has to seek an appropriate mathematical context where such powers of π occur. Perhaps the fundamental volumes of some classical groups furnish that context.

Finally, in dual-string models, the theory simplifies remarkably when working in a lorentzian space with signature (25, 1). This space occurs also in refs 3 and 4 and it is suggested that this numerology may be no coincidence, leading to some physical significance for the "monster" group.

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Magnetic monopoles

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A solitary, uncorroborated event at Stanford is the only evidence that magnetic monopoles might exist. Powerful theoretical motivation for monopoles derives from Dirac's assertion that monopoles could explain charge quantization and the 't Hooft–Polyakov demonstration that monopoles are an inevitable consequence of many gauge theories presently being used to unify the electroweak (photon–lepton) and nuclear (quark) interactions. The monopole abundance suggested by the Stanford event is in clear contradiction to bounds on their number from astronomical data. Fortunately, the already considerable and expanding arsenal of detection techniques are being fashioned to experimentally test the many open questions surrounding monopoles.

THE flush of excitement caused by Cabrera's candidate magnetic monopole event reported in 1982¹ has been reduced to a hush of activity as experimenters around the world contrive and construct detectors. One of their objectives is to corroborate or refute this solitary piece of evidence that is tantalizingly linked with the validity of the current principal theory of matter. This activity has become the focus of many scientists from formerly weakly connected disciplines: low-temperature and high-energy experimentalists, particle theorists, cosmologists and astrophysicists. Such a mixture has produced an excitement characteristic of nascent science before it is subdued into well behaved formality.

Here we review the developments in the field of magnetic monopoles before the Stanford Valentine's Day event to place this important development in context (for an earlier review see ref. 2). Next, the experimental techniques and their results are described. The important question of where monopoles, if they exist, should and should not be found is surveyed. Finally, the implications for theory of the experimental results, both actual and anticipated, are summarized; and opportunities for experiments provided by the development of new theoretical ideas are outlined (see also ref. 3).

History

Perhaps the earliest recorded discussion of magnetic monopoles is found in a letter written by Petrus Peregrinus de Maricourt in AD 1269 during a dreary siege of a rebellious Italian principality; it contains the first glimmerings of the idea of poles and lines of force⁴. The great magnetist, Gilbert, was acquainted with the Peregrinus investigations and Maxwell considered magnetic poles in his unification of electricity and magnetism but lack of experimental evidence caused them to be absent from his final formulation (C. W. F. Everitt, personal communication). In 1931, Dirac⁵, fresh from the triumphant marriage of quantum mechanics and special relativity, turned to a quantum-mechanical study of a problem first addressed classically by J. J. Thomson⁶: the motion of an electric charge in the field of a magnetic monopole. Thomson had noticed the remarkable theoretical fact that the electromagnetic angular momentum in a magnetic pole–electric charge system was independent of their separation. Dirac's result is the now famous quantization condition:

$$eg = n(\hbar c/2) \quad (1)$$

where e and g are, respectively, the electric and magnetic charges and n is the principal quantum number. Stated most dramatically, Dirac demonstrated that a single magnetic pole anywhere in the Universe would explain the fact that all electric charge occurs only as discrete integral multiples of e , in essence

electric charge quantization. The monopole charge is 70 times larger than the electric charge. Two consequences of this large magnetic charge are that a rapidly moving monopole should produce heavy ionization as it passes through matter and that monopoles should bind to some forms of matter. Dirac is moot on other monopole properties: size, shape, mass, parity, spin, statistics, sources, abundance, and so on.

Implicit in all the hopeful but unproductive monopole searches during the ensuing decades was the assumption that the monopole mass was comparable to other particles (for an opposing view, see ref. 7). Such monopoles could attain velocities approaching that of light and could be seen in optical detectors by their bremsstrahlung, de-excitation, Cherenkov, and transition photons. Because of their large energy loss, these monopoles would be brought to rest in matter more readily than their electrically charged counterparts. Once sedentary, monopoles would append themselves to matter from which they could be dragged with sufficiently strong, pulsed magnetic fields. Finally, although these monopoles could be found in cosmic rays, they might also be produced by an accelerator of suitably high energy.

In 1975 one event in a high altitude balloon experiment was claimed to have been produced by the passage of a monopole⁸. Its interpretation was quickly challenged on the grounds of experimental problems, incompatibilities with other work, and possibilities for a less exotic cause (see, for example, ref. 9).

The idea of looking directly for manifestations of magnetic charge using electromagnetic induction was conceived and tested in the 1960s (ref. 10). The passage of a monopole through a closed conducting ring should induce a current change intent on maintaining the ring's pre-monopole magnetic flux. Some of the original experiments were done with room temperature conducting coils. With the advent of superconducting rings in which a current change would persist indefinitely, undegraded by Joule heating characteristic of more mundane materials, these experiments became easier to carry out. The low sensitivity of current-measuring electronics in the early superconducting devices required many passages of a monopole-bearing sample to produce a palpable signal, so this technique was initially restricted to bulk matter searches¹¹. However, with the development of the superconducting quantum interferometer device (SQUID), and ultra-low magnetic field shields, a dynamic monopole detector became possible¹².

Earlier in 1974, a profound theoretical insight was uncovered that would have revolutionary consequences for contemporary monopole searches. Polyakov¹³ and 't Hooft¹⁴ independently showed that monopoles appear as stable solutions of spontaneously broken Yang–Mills field equations and are required by a large class of theories (see, for example, refs 15, 16).

Ironically, the gauge theory evoked in the successful unification of the weak, SU(2), and electromagnetic, U(1), interactions was not one of these, nor was the gauge theory responsible for the also successful quantum chromodynamics, QCD, which described the nuclear force. However, magnetic monopoles do appear in the so-called grand unification theories, GUTs, that unify strong and electroweak forces. Figure 1 schematically illustrates a GUT monopole.

In a typical GUT model there is no difference between strong, weak and electromagnetic forces at a high enough energy or temperature, say in the early Universe, because there is perfect symmetry. This energy is typically 10^{16} GeV. As the Universe expands its temperature falls and the scalar Higgs field starts to acquire a non-zero expectation value. Rapid quenching of the Higgs fields leads to topological defects¹⁷ which are magnetic monopoles whose masses are typically $\sim 10^{16}$ GeV c^{-2} . A proton's mass is ~ 1 GeV c^{-2} .

Such large masses could only have been produced in the first instants after the creation of the Universe in the big bang. Standard cosmology and plausible GUT theory suggest that the number of monopoles is roughly equal to the number of nucleons^{20,21}. On the other hand there is a serious discrepancy. As nucleons account for much of the observed matter in the Universe, there must be less than one monopole per 10^{15} protons. The presence of a galactic magnetic field implies even fewer monopoles²². Thus by 1981, it was argued²³ that the flux of monopoles would be no greater than 10^{-16} cm⁻² s⁻¹ sr⁻¹.

Because GUT monopoles are expected to be very massive and therefore not heavily ionizing, they would be slow, would be hard to stop, and if somehow trapped in matter, would easily be dislodged by even modest accelerations. For example, such a monopole moving at 1/100 of the speed of light could easily penetrate the earth. The most powerful electromagnet, 1 km long, would deflect the monopole less than 10^{-8} of a degree. Thus, this one idea explained why none of the experiments since Dirac's prediction would have seen a magnetic monopole. Further, it left only one detection technique free of questions—that used by the dynamic induction detector.

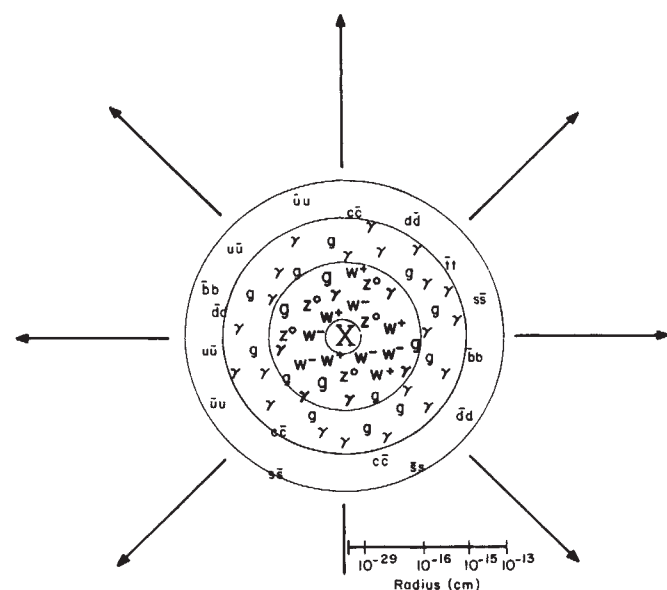


Fig. 1 The GUT monopole is structured in regions defined by the degree of unification and virtual constituents. From the inside-out there is a grand unification core where all the GUT gauge particles including X bosons are present. The electroweak unification region includes intermediate bosons (Z, W), the confinement region only has photons (γ) and gluons (g), and a fermion-antifermion condensate.

The candidate Stanford event of February 1982 was recorded in such an induction detector. The event possessed characteristics consistent with the passage of a particle of magnetic charge g to $\pm 5\%$ (ref. 1). The instrument which detected this monopole candidate, shown in Fig. 2, was a magnetically shielded magnetometer. The superconducting detector loop was shielded with a sophisticated superconducting shield that held ambient magnetic fields to $<10^{-7}$ G, among the lowest ever obtained. Baseline noise in the Cabrera system was typically 1% of a monopole offset signal. Although this signal is not easily attributable to any other probable cause, its possible origin in the release of some internal instrumental stress cannot be entirely ruled out. Without a second event being seen to date, this experiment has now²⁴ set an upper limit of 4×10^{-11} monopoles cm⁻² s⁻¹ sr⁻¹ for the isotropic distribution of particles of charge >0.06 g. This is 10^5 times larger than the earlier expected upper limit²³.

An induction detector has been used to examine the superconducting niobium spheres¹² on which Fairbank *et al.*²⁴ have reported finding fractional electrical charges. Measurements made over a decade on the niobium spheres have failed to reveal any magnetic charge. These searches were motivated by Schwinger's proposal²⁵ in the mid-1960s that combined both electric and magnetic charge into an object called a dyon. Experimental searches for dyons have been equivalent to those for magnetic monopoles.

Detectable properties

The presence and properties of a monopole can be deduced from their unique interactions with matter. Most techniques that are used to detect electrically charged particles have some problems for GUT monopoles so first we shall discuss an approach which can uniquely detect superheavy monopoles.

Induction: When the magnetic flux through a conducting ring is changed, a current is induced in the ring. This current is quickly extinguished by the resistance of the ring material except for a superconducting ring where the macroscopic quantum mechanical effect described by the Ginzberg-Landau formulation occurs. (Gorkov²⁶ demonstrated that the Ginzberg-Landau

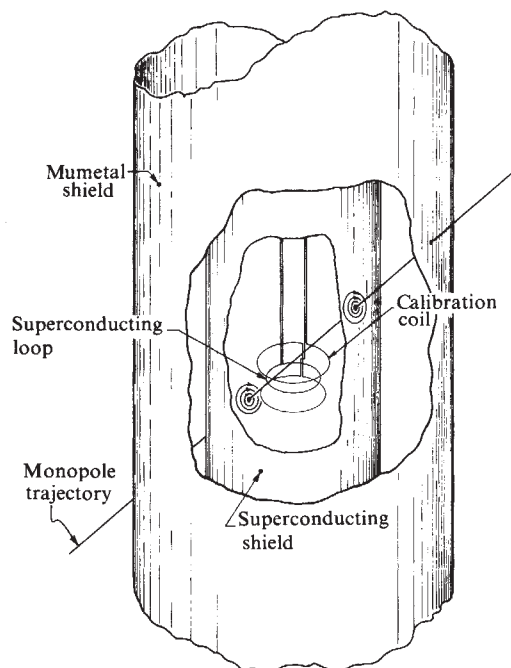


Fig. 2 Schematic diagram of the Stanford dynamic monopole detector that found the candidate monopole signal. This ultra-low field superconducting magnetometer is a four-turn 5-cm diameter niobium wire ring positioned with its axis vertical. The ring, connected to a SQUID, is mounted inside an ultra-low field shield which, in turn, is mounted inside a single mumetal cylinder to provide 180 dB isolation from external magnetic field changes (taken from ref. 2).

macroscopic description of superconductivity follows from the Bardeen-Cooper-Schrieffer microscopic theory.) The supercurrent density is

$$\mathbf{j}_e = \frac{he^*}{2im^*} (\psi^* \nabla \psi - \psi \nabla^* \psi) - \frac{e^* c}{m^*} \psi^* \psi \mathbf{A} \quad (2)$$

where the particles involved are Cooper pairs whose mass and electric charge are twice that of the electron and $\mathbf{A}$ is the electromagnetic vector potential; ψ , the coherent many-body state of Cooper pairs, has a local pair density, $\psi^* \psi = n_s/2$, which is half the superelectron density, n_s . This equation can be solved² in conjunction with Maxwell's equations for a closed loop to show that the magnetic flux through the ring must be an integer number of

$$\phi_0 = (hc/2e) = 207 \text{ nG cm}^2 \quad (3)$$

the superconductivity flux quantum. For a magnetic monopole passing through a ring the flux coupling the ring has two components: the self-induced ring flux which is the supercurrent times the ring self-inductance, L , and the monopole flux coupling the ring which depends on the monopole velocity, v ($\beta = v/c$ and $\gamma = (1 - \beta^2)^{-1/2}$), and the ring radius, R ; c is the velocity of light. The induced ring current is

$$I(t) = (\phi_0/L)(1 + \gamma vt((\gamma vt)^2 + R^2)^{-1/2}) \quad (4)$$

The process by which a monopole pulls flux through the loop to induce a toroidal field around it is shown in Fig. 3. The characteristic time, $R/\gamma v$, is ~ 100 ns for $\beta \sim 10^{-3}$ and R of a few centimetres. For particles passing through a ring, persistent current change will only be produced by a magnetic charge. The sign of the current depends on the polarity of the magnetic charge and its direction of passage through the loop. An electric charge or magnetic dipole, as well as a magnetic charge missing the ring, will cause only small, transient current changes.

Since a changing ambient magnetic flux through the loop will also induce a current change, superconducting shields are used to freeze the ambient field in place. The shield also interacts with the loop and reduces the offset current depending on its distance from the loop. Cabrera's candidate event could have been caused by an ambient flux change through the ring although his superconducting shield is the best in the world. Other induction searches using gradiometers²⁷ or macromes (H. Frisch, personal communication) have opted for less sophisticated shielding in order to make larger rings. Most of these detectors, such as Cabrera's currently operating triaxial detector, incorporate multiple loops for redundancy, possible direction measurement, and increased detection area. Non-superconducting techniques have also been discussed (ref. 28 and D. Morris, personal communication). The combination of a superconducting loop, shield and SQUID appears in practice to provide the best sensitivity. Cabrera has also suggested scanning the shield with a sensitive magnetometer to detect the twin magnetic vortices left by the passage of a magnetic monopole.

Ionization: Several processes involving electromagnetic interactions of electrically charged particles result in detectable photons and concomitant energy loss for the particle. The analogue interactions for magnetic monopoles benefit from the stronger magnetic charge but suffer from the expectation that a massive GUT monopole will have a velocity much smaller than that of light.

Detailed calculations of the photon-producing processes for magnetic monopoles conclude that atomic excitation holds the most promise for monopole detection. Current energy loss estimates for GUT monopoles with β of 10^{-3} differ by several orders of magnitude^{29,30,74,75}. This uncertainty has a bearing on the possibility of detecting monopoles in various media and for trapping them in astronomical bodies since their range will depend on their energy loss rate. On the other hand, ionization detectors can be made extremely large.

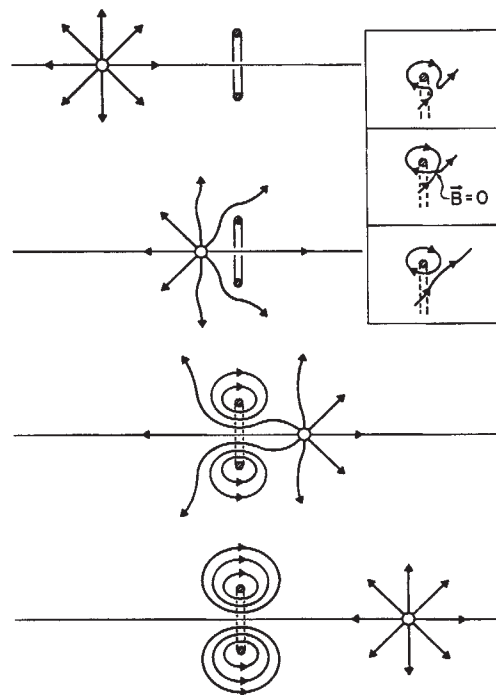


Fig. 3 Effect of a monopole passing through a current loop. The monopole effectively draws flux through the loop and leaves a residual toroidal field around it. The inset shows how the flux lines around the loop pinch off to form closed circles (taken from ref. 3).

The origin of the uncertainty in energy loss estimates lies in the fact that atomic collisions are complicated. For relativistic charged particles, these inherent complications are simplified by arbitrarily separating the interaction into two soluble classes: close collisions and distant collisions. In close collisions the energy transfers are so much larger than the electrons's binding energy that the electron is considered free and the impulse approximation is satisfactory. The energy loss is then easily calculated from simple kinematics and the scattering cross-sections. In distant collisions, the atom is considered to be excited by the perturbing electric field of the glancing particle and the dipole approximation is used.

These calculations break down when the velocity is so small that most of the collisions no longer fall into only one class; then a real model for the atom must be used to understand the dynamics. The actual calculation requires approximations to produce a result. Some years ago, Lindhard³¹ worked out this theory for protons. Experiments done with protons down to β values of $\sim 10^{-3}$ are in good agreement with theory. Recently, energy loss to Zeeman splitting at low velocities has been studied from a fundamental basis³². Estimates of energy loss at low velocity to this mechanism in helium and hydrogen are substantially higher than ionization losses in silicon. Figure 4 summarizes the situation for the estimated energy loss.

An interesting possibility is a combination of an induction and ionization detector. If a monopole passes through such a detector and no ionization is detected within a few milliseconds of the induction event, the credibility of the ionization loss estimates and/or induction signal is called into question. Perhaps the monopole velocity is much slower than previously thought, implying an even heavier monopole. If attendant ionization is discovered, the monopole speed and direction could be determined to give the polarity of the magnetic charge.

Ionization experiments which use either plastic scintillation counters coupled to photomultiplier tubes or proportional ionization chambers typically have several detector planes spaced metres apart to signal the coordinates and time of passage of a monopole candidate event. For GUT monopoles these signals would be separated in time by microseconds. Cosmic ray events are the overwhelming source of background, so placing

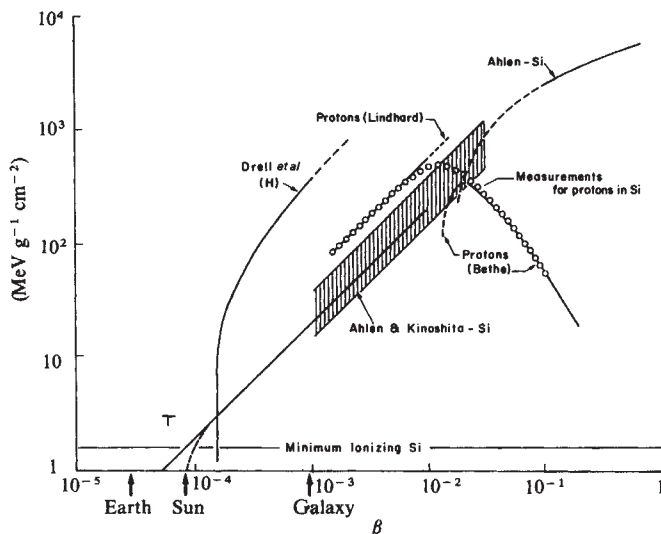


Fig. 4 Predicted energy loss rate to silicon for protons and magnetic monopoles as a function of β . The lines represent predictions while the circles are averages of proton measurements. The hydrogen calculation includes the effect of Zeeman splitting. A threshold, T , as shown for silicon, can cut off the energy loss. Reference escape velocities from the Earth, Sun and Galaxy are shown (refs 29–32).

the detector deep underground should reduce this background. At lower velocities the energy loss is expected to be smaller, so there is a premium on making the detectors sensitive to small ionization losses.

Acoustical: Over a decade ago, Hofstadter³³ suggested and demonstrated experimentally that electron beams could produce mechanical oscillations and a detectable thermoacoustic wave. Despite considerable interest in the possibilities, no particle detector has been developed using this principle due to the poor signal-to-noise ratio. Encouraged by early estimates of monopole energy loss of 2 GeV cm^{-1} (ref. 34), interest in this possibility for monopole detection has been renewed. However, calculations of the thermal fluctuation pressure suggest that this severely limits the thermoacoustic detection of monopoles for conductive media with temperatures above a few millidegrees³⁵. Noise sources attendant on actual acoustic measurements are also discouragingly large. Experiments are being done to explore acoustical detection³⁶.

Electromagnetic: During the 1950s, five emulsion events taken in balloon flights had the unusual property that only electron-positron pairs in great number were present in a configuration which indicated a very energetic process—certainly above a few TeV—and showed no track of a causal particle³⁷. These unusual events have been interpreted as photoproduction of a virtual monopole-antimonopole pair with the resulting bremsstrahlung and annihilation radiation being visible³⁸. Accelerator searches have failed to discover similar events².

With the advent of the GUT monopole, the monopole-antimonopole system (now familiarized to monopolonium) would have been revisited³⁹. Monopolonium would have some remarkable properties. At a separation of $1/10$ Å the lifetime of monopolonium would be as long as 10^{11} yr. At a separation of 1 Å , the velocity of the monopole in orbit is 1 cm s^{-1} , the principal quantum number is roughly 10^{12} , but the binding is still 40 keV . Monopolonium would decay by classical Larmor radiation for all but the last 10 seconds of its life. It de-excites first by emitting radio, then successively light, X rays, γ rays, quarks and gluons, intermediate bosons, and ultimately $10^{14} \text{ GeV c}^{-2}$ X, GUT bosons. Typically some tens of millions of particles would be emitted altogether. Monopolonium radiation at a wavelength of 1 cm might produce a flux of $10^{-24} \text{ eV}^{-1} \text{ cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$. Current observational limits are $3 \times 10^{-16} \text{ eV}^{-1} \text{ cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$, corresponding to $50 \mu\text{Jy}$. In general, the prospects for other monopolonium products are equally dim.

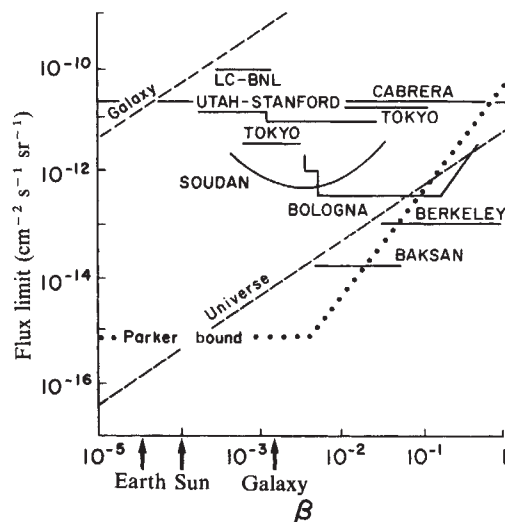


Fig. 5 Limits for cosmic-ray flux of GUT monopoles (95% confidence limits) plotted against the β of the monopole. The Stanford result corresponds to one candidate event. All other detectors relied on ionization. Also shown for monopoles whose mass is 10^6 GeV c^{-2} are the Parker galactic bound (····) and upper limits based on the expansion of the Universe for monopoles distributed uniformly and clumped in galaxies (----). (Taken from refs 42, 66–73, and B. Cabrera, personal communication.)

Sources and sinks of monopoles

Possible sources and sinks of GUT monopoles (with relevant problems) include: the big bang (inaccessible), monopolonium (rare), the Galaxy (extant magnetic fields), the surface of the Earth (improbable), the Sun (unlikely), the interior of the Earth (less likely than the Sun), meteorites (braking problems), accelerators (too puny), cosmic rays (rare), made by cosmic rays (still too puny), in detectors searching for proton decay terrestrially, and in neutron stars (theoretical reservations on monopole catalysis). These monopole sources and sinks are considered, moving from the nearby space-time to the big bang.

Accelerator searches: Traditionally, particle searches are most successfully carried out at accelerators. Because of the unusual properties of monopoles, it is possible to devise accelerator experiments that are background-free. No monopoles have been found in either fixed-target or colliding-beam accelerator searches⁴⁰. Indeed, in all cases, these cross-section limits are typically the smallest measured at an accelerator. However, such searches can shed no light on the GUT monopole. Gauge theories with monopoles suggest a lower limit on the monopole mass⁴¹, which is greater than the mass of the gauge boson associated with the theory divided by the appropriate coupling constant. For an intermediate boson mass of 80 GeV c^{-2} and the fine-structure constant, the monopole mass would be $10,000 \text{ GeV c}^{-2}$. (Note, however, that the standard electroweak theory does not contain a monopole.) No known accelerator technology can reach the energies of 10^{16} GeV needed to produce grand unification monopoles. Thus, accelerator monopole searches are no longer in the mainstream, but they will continue to be done as other unexpected production mechanisms might arise for monopoles that do not derive from grand unification.

Cosmic ray searches: The total cosmic ray flux at the surface of the Earth is $0.01 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$. The present reported Cabrera flux limit is equivalent to a hundred-millionth of this. Astrophysical estimates of monopole flux limits²³ suggest values less than $10^{-15} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$. Such fluxes require detectors the size of a football field and which operate for years. The largest area-time factor reported for an ionization search is from the Baksan neutrino detector⁴², which has produced a limit on the flux nearly 10^4 times lower than the current Cabrera limit. These limits are summarized in Fig. 5.

Cosmic rays can also produce elementary particles through interactions. Although cosmic ray energies range up to

10^{11} GeV, this is still too small to produce GUT monopoles. Thus, GUT monopoles found in cosmic rays would have to be primordial.

Matter searches: Monopoles could be present in matter either because they were accreted there when the matter formed or because they stopped after losing their kinetic energy. Monopoles could bind to ferromagnetic or paramagnetic material through image charges^{43,44}, and possibly to atomic nuclei⁴⁵. Typically in planetary formation they would reside near the core since the force of gravity would overcome any sustaining force of matter, and gravitational forces are more significant than magnetic forces.

An obvious possibility is to search iron from the surface of the Earth. However, the expected kinetic energy of a cosmic GUT monopole is such that it could stop anywhere in the Earth's interior, so that the number of monopoles should be small and uniform through the Earth. One suggestion is to look for monopoles falling under gravity out of iron refinery operations⁴⁵, where millions of tons of surface ore are raised above the Curie point every year in the manufacture of steel. In another approach, the Earth's heat flux has been used to obtain limits on monopoles buried deep in the core^{46,47}.

Solar System sources: Meteors, with their smaller gravity, might provide a safe harbour for monopoles⁴⁸. Unfortunately, a meteor-trapped monopole that hits the Earth will continue unimpeded because it carries far more momentum than the normal atoms and would shear right through matter.

The Sun has also been suggested as a source of monopoles⁴⁹. This scenario evolved principally to explain the unexpectedly high flux suggested by the Cabrera event. In this picture the Sun contains 10^{26} monopoles and emits 10^9 monopoles per second during its lifetime. Solar flare fields expel monopoles with velocities similar to the Earth's velocity around the Sun, that is, 30 km s^{-1} (one-fifth of the typical galactic velocities), so that they form a cloud in the Earth's orbit. This model may be alone in accommodating an 11-yr sunspot cycle. However, the concentrating effect for GUT monopoles in the Sun has been calculated to be well below that needed to achieve the posited solar population⁵⁰, suggesting that a solar monopole level of this magnitude would have to be primordial.

A few observations of the Sun suggests that it has a magnetic monopole moment⁵¹. Taken at face value, these measurements are consistent with a net north monopole abundance of $\sim 10^{29}$ (L. W. Alvarez, personal communication). Many other more prosaic explanations have been offered for these observations⁵²⁻⁵⁴.

The current view of the GUT monopole raises the possibility that when it passes through the nuclear matter, collisions may cause protons and neutrons to spontaneously decay. In other words, baryon decay, which must ordinarily be exceedingly rare, may be catalysed in the presence of a monopole. Although the possibility of baryon catalysis^{18,19} has attracted much interest, there are still many questions concerning the details.

Monopole baryon catalysis offers an interesting possibility for terrestrial observations. In SU(5) grand unification, the proton lifetime has been predicted to be $\sim 10^{31}$ yr so that its detection requires large amounts of matter. On the other hand, a monopole passing through a proton decay detector could catalyse with a strong interaction cross-section, several decays. The upper limit on the monopole flux at the Earth based on the catalysis possibility is currently $< 5 \times 10^{-15} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ in the absence of any observed events and assuming the catalysis cross-section to be $\sim 100 \text{ mb}$ (refs 55, 56).

Astrophysical sources: Neutron stars should be relatively good monopole collectors⁵⁷. Once inside a star, catalysing monopoles could transform nucleons at such a rate that the resulting X-ray luminosity would exceed by many orders of magnitude the measured upper limits of neutron star X-ray luminosities. This implies a monopole flux, assuming hadronic cross-sections for catalysis, of $< 5 \times 10^{-22} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$, much lower than any other flux limit. There are still questions about the heat transfer mechanism inside a neutron star, the catalysis rate, the micro-

environment surrounding a monopole, possible recombination of monopole pairs and the superconducting interior of a neutron star which have a bearing on this argument. A non-catalysis limit of approximately the same level has been set using the millisecond pulsar (ref. 58 and J. A. Harvey, personal communication).

Monopoles are sometimes suggested as the source of the 'dark matter' which appears to be necessary to describe the dynamics of the galaxy and other large astrophysical clusters. Monopoles could supply such mass without producing much radiation.

Central to the question of galactic magnetic monopole abundance is the Parker bound²². Free monopoles in a magnetic field will neutralize the field since the electric currents that generate the field have to do work on the monopoles, thereby dissipating the currents. This implies that the flux limit for free monopoles is less than $10^{-16} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$, or roughly 10^5 times smaller than the Cabrera flux. This is, of course, the flux for free monopoles not bound in some way or near a monopole source.

Recently, this limit has been re-examined in detail as a function of monopole mass and velocity⁵⁹. Even for monopoles having masses of $10^{19} \text{ GeV } c^{-2}$, near the Planck mass, it is difficult to achieve fluxes higher than $10^{-12} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$.

One escape from the Parker bound could be that the galactic magnetic field is due to monopoles^{60,61}. If the galactic field were produced by magnetic monopoles, however, it should have a vanishing curl, which contradicts the observation. Indeed, the magnetic field configuration and magnitude generally fit the picture of a dynamo. The relatively short field oscillation time for a monopole galactic field would prevent dust grain alignment⁶², a process known to exist in the Galaxy from star light polarization measurements.

Parker bound arguments can be extended to the impact of monopoles on extragalactic magnetic fields. There is evidence for intragalactic cluster fields of the order of 10^{-7} G . These low fields can be used to infer flux limits in our Galaxy a thousand times lower than the galactic Parker bound⁶³.

The big bang: The conundrum of monopoles in the big bang has already been mentioned^{20,21}. Standard cosmology and SU(5) GUTs suggest a monopole number roughly the same as the proton number in the Universe. The estimated total protonic mass in the Universe is not far from that needed to close the Universe. If an equivalent number of monopoles with their enormously large mass is added, the Universe would have closed far too quickly. This implies that there cannot be as many monopoles as naive cosmological estimates would indicate.

Of the ways to reduce the number of monopoles, perhaps the most intriguing is the inflationary universe hypothesis⁶⁴: in essence, the Universe expands exponentially and supercools. The actual expansion is enormous. This process erases the previous history of the Universe. After the phase transition occurs, the universe reheats and evolves along the lines of the standard big bang. With one stroke this model can severely depress monopole production⁶⁵ and explains the flatness and the horizon problems. However, the inflationary scenario has not been satisfactorily implemented in any realistic GUT model.

The future

The Cabrera event has stimulated several inductive searches, the most unambiguous way to detect a monopole. However, none of these has seen a second event so that the flux limit is now an order of magnitude lower than in 1982 and may well be an additional 100 times lower in a year or so. This is still 10,000 times the Parker limit.

The recent theoretical work on ionization of monopoles in matter has placed ionization experiments on a firmer footing down to β values of 10^{-3} . Uncertainties in ionization experiments are balanced by larger detection areas. If the many large-scale searches now underway give null results, then the largest can come close to the nominal Parker bound.

The best opportunity to answer monopole questions may lie with the grand scale of cosmology and astrophysics. Most inter-

esting might be evidence of some large-scale artefact that could be definitely linked to the existence of monopoles during some period in the evolution of the Universe.

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ARTICLES

3CR249.1 and Ton202—luminous QSOs in interacting systems

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The morphologies of the extended emission-line regions around the QSOs 3CR249.1 and Ton202 indicate that they have, in both cases, resulted from interactions between two galaxies of roughly equal mass and that the galaxies involved possessed extensive rotating gaseous disks before their encounters. These two examples provide support for the position that luminous QSOs are frequently activated by violent interactions. More generally, the distribution of gas around luminous QSOs promises to be a sensitive and useful indicator of the recent history of their physical environment.

THE idea that certain types of nuclear activity in galaxies may be triggered by interactions with other galaxies was suggested by Toomre and Toomre¹ in their classic study of galactic bridges and tails. Addressing more specifically the kind of activity seen in QSOs, Gunn² pointed out the difficulty of providing, through normal internal processes in present-day galaxies, the mass flow into the nucleus necessary to fuel the observed luminous output of bright QSOs and suggested that some such external disturbance might actually be a requirement for the most luminous forms of nuclear activity. Recently, claims of observational support for interactions involving QSOs have been made³⁻⁶, but the cases involving 'classical' high-luminosity QSOs are

ambiguous and subject to other possible interpretations, while unequivocal cases of interaction among low-luminosity 'QSOs' extend to levels of nuclear activity only slightly greater than well-known examples among Seyfert galaxies⁷. We present here two cases of high-luminosity QSOs for which the morphology of extended ionized gas provides strong evidence for interaction.

Emission-line regions extending to a few arc s (typically 10–30 kpc) radial distance have been found around the QSOs 3CR48 (ref. 8), 4C37.43 (ref. 9), and 3CR249.1 (ref. 10), as well as a number of lower luminosity objects^{6,11-13}. For 3CR48 and 4C37.43, the distribution of the emitting gas is strongly asymmetrical with respect to the QSO nucleus; this

asymmetry and the detection by Boroson and Oke¹⁴ of an early-type stellar population in 3CR48 have led us to speculate^{3,15} that this gas may be debris from recent tidal interactions, but the detailed structures in these two cases have not yet yielded any simple interpretation. The spectrophotometric technique used by Richstone and Oke to detect the extended emission around 3CR249.1 did not allow the distribution of the gas to be determined. The fact that the redshift of 3CR249.1 ($z = 0.312$) places [O III] $\lambda 5,007$ (the strongest line seen in the extended emission regions around 3CR48 and 4C37.43) conveniently close to the rest wavelength of $H\alpha$ led us to attempt to image it at this wavelength through a 30-Å bandwidth interference filter in order to obtain high contrast against the sky background. We have since acquired 30-Å bandwidth interference filters at other wavelengths and begun a survey of a number of other luminous QSOs. Of the 15 new objects imaged so far, four show obvious extended [O III] emission. In two of these cases, the emission is weak and apparently confined to a single region centred a few arc seconds from the QSO; in one case, it is strong but shows a very complex morphology, somewhat like that associated with 4C37.43. The remaining case, Ton202, shows a structure similar to that of 3CR249.1, and here we discuss imaging and spectroscopy of both objects.

Imaging

All images were obtained with the Galileo/Institute for Astronomy CCD (Charge Coupled Device) system¹⁶, using a Texas instruments 500×500 pixel, three-phase, backside-illuminated detector, mounted either on the University of Hawaii 2.2-m telescope or the Canada-France-Hawaii 3.6-m telescope. The [O III] $\lambda 5,007$ images were obtained with 30 Å FWHM interference filters centred by tilting to within ~ 3 Å of the best available wavelength for the line in the spectrum of the QSO. In addition, exposures in adjacent line-free continuum regions were obtained through moderate bandpass filters.

3CR249.1

Figure 1a shows the [O III] image of 3CR249.1: the QSO is centred in an extended envelope aligned roughly north-east-south-west, and two elongated filamentary structures are seen, one proceeding westward from the northeastern end of this envelope, the other, strongly curved, coming from a discrete condensation centred 3 arc s south-east of the QSO. Assuming (as we do throughout) that $H_0 = 75$ and $q_0 = 0.5$, the scale is $3.75 \text{ kpc arc s}^{-1}$, giving a maximum projected span across the detected emission regions of 55 kpc. Figure 1b shows an image obtained in the line-free continuum from 5,070 to 5,370 Å in the rest frame of the QSO. It is clear that there is no substantial amount of continuum radiation associated with any of the extended emission-line features. In particular, we can set an upper limit of $m_R \sim 23.5$ to the continuum brightness of the condensation seen 3 arc s south-east of the QSO in Fig. 1.

We have obtained spectra at a nominal dispersion of 100 Å mm^{-1} of 3CR249.1 and various points in the surrounding emission regions with the University of Hawaii 2.2-m telescope standard Cassegrain spectrograph and the RCA C33063 photographic image intensifier system. Some of the spectrograms were degraded by instrumental flexure, the effect of which has been minimized by using profiles of airglow lines from the same spectrograms as templates for determining emission-line redshifts.

All relative velocities are based on measurements of the [O III] $\lambda 5,007$ line: not only is it the easiest feature in the spectrum to measure, but there is reason to believe that the redshift of the narrow-line region in a QSO may give the best available estimate of the systemic velocity¹⁷. Standard deviations of velocity differences are estimated to be typically 30 km s^{-1} .

Observed radial velocities, relative to the rest frame of the QSO, are as follows: the condensation to the south-east, -40 km s^{-1} ; the southeastern tail, -7 km s^{-1} ; the tip of the northwestern tail, 27 km s^{-1} ; the inner northwestern tail (3 arc s from the QSO), 15 km s^{-1} ; and a point in the envelope 3 arc s

north-east of the QSO nucleus, 96 km s^{-1} . Only this last velocity difference can be considered significantly different from zero; however, the fact that no high velocities were observed anywhere in the system is itself remarkable and may be taken as testimony that a luminous QSO can occur in a system of quite modest mass.

Ton202

Figure 2 shows the images of Ton202. Two opposing arms or tails are seen in the [O III] image, the stronger one extending to the east. With a scale of $4.1 \text{ kpc arc s}^{-1}$, the projected tip-to-tip distance across the emission features is 38 kpc. Unlike 3CR249.1, Ton202 shows definite extension in the continuum image (inset in Fig. 2), on the western side near the base of the weaker [O III] feature. The peak pixel in both the emission-line and continuum images in Fig. 2 is indicated in order to show this asymmetry. In the spectrum of Ton202 shown in Fig. 3, the continuum extension can also be seen as a less steep gradient on the western (bottom) side. This spectrum was obtained with the same CCD system and focal reducer as the images, with the addition of a slit in the telescope focal plane and a zero-deviation grism in the collimated beam of the focal reducer. Images of the slit and the field were recorded immediately after the spectroscopic exposure, allowing corrections to be made to the velocities for uneven illumination of the slit.

The [O III] $\lambda 4,959, 5,007$ lines give a redshift for Ton202 of 0.3630. Relative to the QSO, the condensation at the end of the eastern tail is approaching, having a velocity of -72 km s^{-1} ; the tip of the fainter (western) tail is receding, with a velocity of 211 km s^{-1} ; and the bright emission near the base of the western tail has a velocity of 132 km s^{-1} .

Interpretation

Recent detailed studies of two radio galaxies with surrounding emission-line regions by Fosbury and his collaborators^{18,19} have shown that the extended emitting gas is probably debris from recent mergers. We believe that a similar origin holds for the cases of 3CR249.1 and Ton202. The [O III] image of 3CR249.1 (Fig. 1a) is reminiscent both of the numerical simulations of interacting galaxies by Toomre and Toomre¹ and of certain local cases of such interactions, such as NGC2623 and NGC4676. While it might be possible that elongated features may be produced by ejection or by entrainment of thermal material at the boundaries of radio jets, we believe that such explanations cannot apply to the present observations of 3CR249.1. The curvature of the southeastern tail and the fact that the north-western tail does not point towards the nucleus would seem to rule out ejection, and examination of a 6-cm B-array VLA map, shown to us by J. Wardle, does not show any evidence for correspondence between the radio and optical structure, even though the brightness peak in the eastern lobe lies near the end of the curved tail.

The straight extension to the north-west of 3CR249.1 is easily visualized as a curved tail viewed from the side, the increased luminosity at the tip being perhaps due to a line-of-sight tangential to a portion of the curve, much as the southeastern tail would appear if it were viewed from the north-east or south-west in its own plane. If this is the case, and if the elongated distribution of gas around the QSO is a disk inclined at roughly 60° to the plane of the sky, the passage of the companion must have been one of reasonably high inclination in order to produce a tail that apparently deviates substantially from the plane of the disk. Because of unresolved ambiguities in interpreting the true three-dimensional structure of the system, it is difficult to say more without better velocities, but that the observed extended structure is of tidal origin seems beyond serious doubt.

In some respects, however, the close similarities between Fig. 1 and typical illustrations of nearby interacting pairs are potentially misleading. It is important to remember that one is comparing an emission-line image on the one hand with images dominated by stellar continuum radiation on the other. In the tails associated with 3CR249.1, there is no evidence that any substantial amount of star formation has taken place; the fact

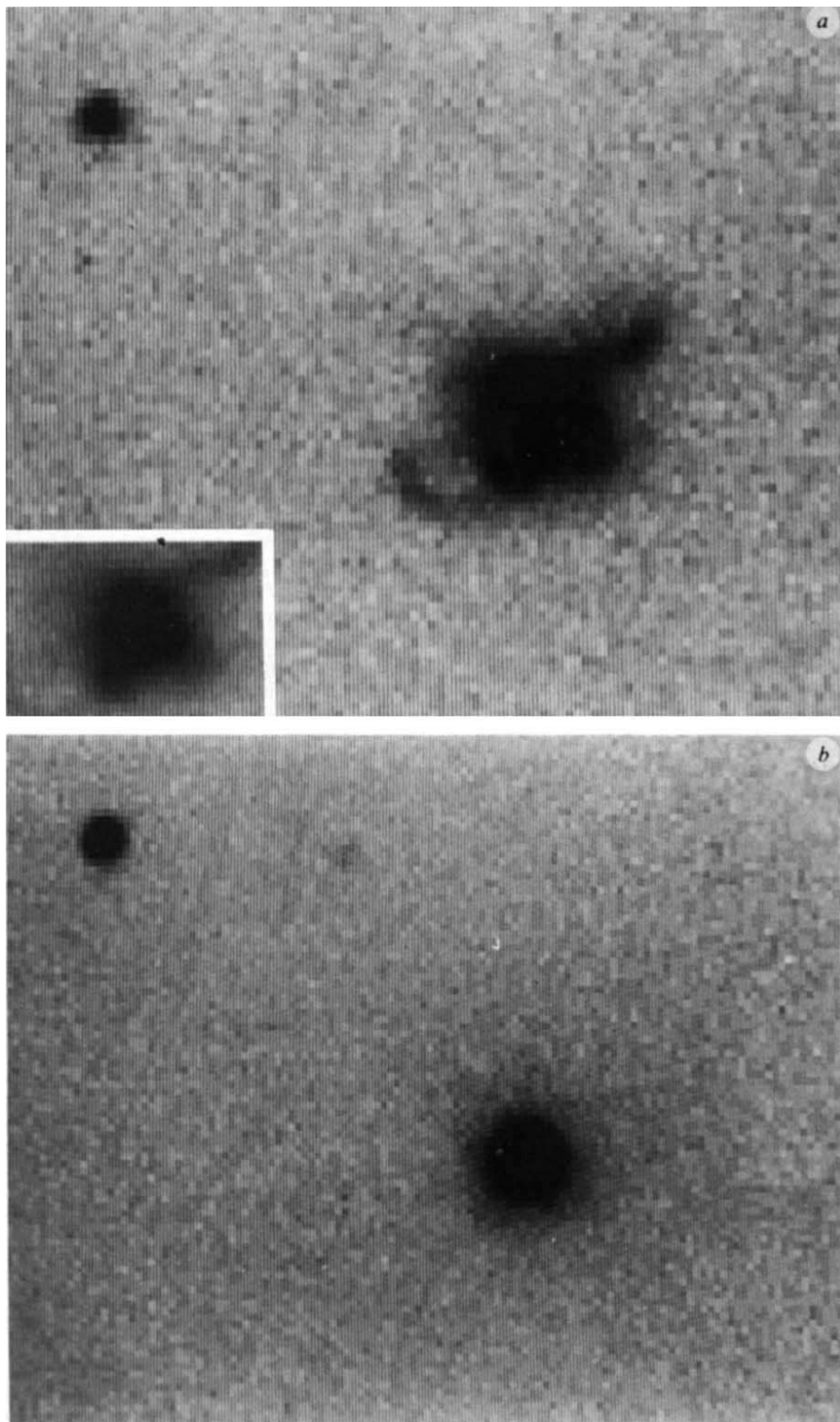


Fig. 1 *a*, Image of 3CR249.1 through a 30-Å bandwidth filter centred on the redshifted [O III] $\lambda 5,007$ line. North is at the top and east is to the left. The data values for this image as well as those shown in Figs 2 and 3 have been transformed to a logarithmic scale in order to display the entire range while retaining discrimination of small differences near the sky background. The area displayed is 40×32 arc s. The inset shows the QSO image at lower contrast. This image was obtained with the UH 2.2-m telescope; all others were obtained with the CFH telescope. *b*, A corresponding image of 3CR249.1 in the line-free continuum from 5,070 to 5,370 Å in the frame of the QSO.

that they are detectable at all is entirely due to their being photoionized by the far UV continuum from the QSO²⁰. Such gas would be detected in quiescent nearby interacting galaxies only by 21-cm observations. Thus, interactions producing signatures (such as tails and bridges) involving amounts of gas insufficient to produce appreciable star formation and which would not ordinarily be apparent can become so in the presence of a luminous active nucleus.

The presence of two well-defined, fairly narrow tails associated with 3CR249.1 indicates two things: first, that both of the participants possessed rotating gaseous disks before their encounter, that is, they were probably spiral galaxies; and second, that the mass ratio of the two systems cannot be too

different from unity¹. Can we identify the interacting companion galaxy? Looking at Fig. 1*a*, one has a strong temptation to place the companion at the position of the bright emission condensation 3 arc s south-east of the QSO. The upper limit to any continuum radiation from this region translates into an absolute magnitude fainter than $M_V \sim -17.5$. As we have already inferred from the low observed velocities, the total mass involved is small, so this conjectured position for the companion does not violate any of the available observations. We emphasize, however, that it would not be very surprising if the bulk of the mass of the companion were not coincident with the local peak in the [O III] emission south-east of the QSO. In the case of the active galaxy 0351+026, where the interacting companion

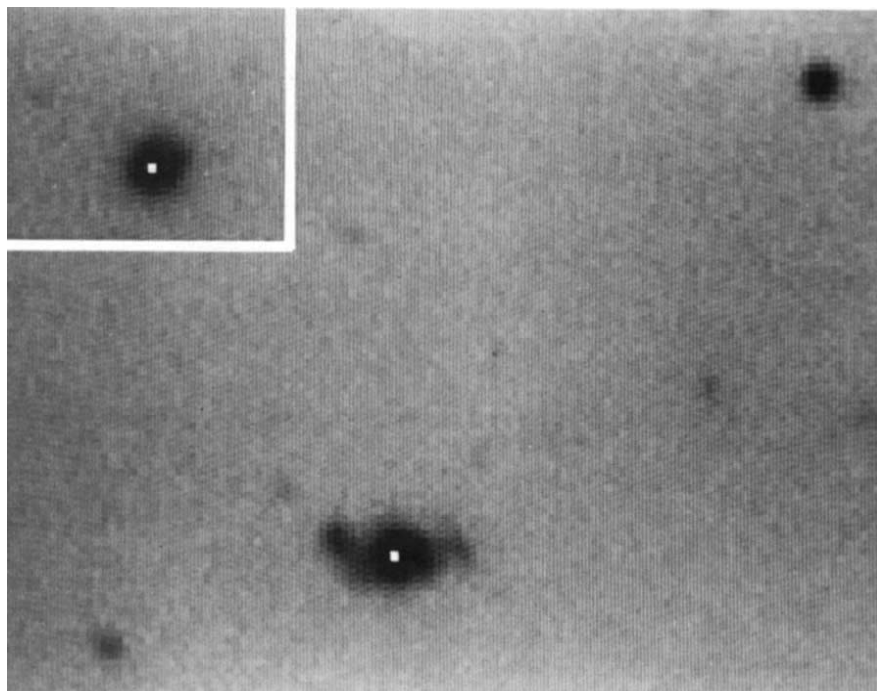


Fig. 2 Image of Ton202 through a 30-Å bandwidth filter centred on the redshifted [O III] λ 5,007 line. North is at the top and east is to the left. The area displayed is 64×46 arc s. The inset shows an image of Ton202 in the line-free continuum from 4,700 to 4,830 Å in the frame of the QSO. The peak pixel of each image is marked in order to show the asymmetry in the images.

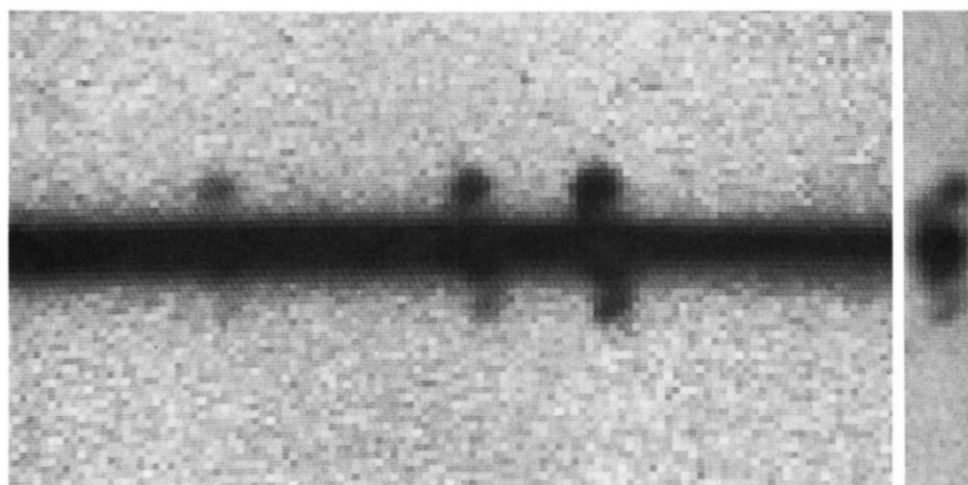


Fig. 3 Spectrum of Ton202, showing H β and the [O III] $\lambda\lambda$ 4,959, 5,007 emission lines. An [O III] image of Ton202 is shown at the same scale on the right. The slit was centred on the bright condensation in the eastern (upper) tail and had a projected width of 4 pixels. Note the difference in the gradients on the two sides of the continuum, indicating resolved continuum structure on the west side of the QSO.

is clearly visible, the peak in the [O III] emission is displaced from the stellar distribution⁶, and a similar effect seems to be present in Ton202, where the elongations of the [O III] and continuum images in Fig. 2 seem to differ in direction by about 30°. Thus it is quite possible that in 3CR249.1 the companion is either projected closer to or merged with the galaxy in which the QSO resides (compare with NGC7252, which still shows a pair of well-developed tails even though the main disks of the galaxies have merged into a single entity²¹).

The morphology of Ton 202 is less blatantly suggestive of tidal interaction than that of 3CR249.1, but two key ingredients are still present: the considerable asymmetry in the surface brightness of the two tails, and the off-centre continuum 'bump' opposite the base of the brighter tail. Another indication that we are not simply seeing an uneven distribution of gas associated with an otherwise normal single galaxy is the asymmetry in the velocities at similar distances on either side of the QSO, which would make sense if the eastern tail is associated with the QSO and the western tail with a companion receding from us relative to the QSO.

Conclusions

The clearest previous example of an interaction involving an object regarded as a QSO is that of 0351+026^{5,6}; but if 0351+026 can be called a QSO, it achieves this distinction largely by

default: only a modest level of nuclear activity is required to overwhelm the low-luminosity galaxy in which the nucleus resides. In terms of the absolute luminosity of its active nucleus, 0351+026 lies below the middle of the usual Seyfert range, some three magnitudes fainter than Seyferts such as 3CR120, Mkn231 and NGC7469. The special importance of 0351+026 lies not so much in its being an active nucleus in an interacting system (although it remains a particularly clear and pleasing example) as in demonstrating that an active nucleus can exist in a dwarf galaxy.

As we have indicated, 3CR249.1 also seems to be in a low-mass galaxy, but both 3CR249.1 and Ton202 have luminosities over a hundredfold greater than that of 0351+026, placing them near the upper end of the QSO luminosity function. Because the luminosity of such nuclei in most cases frustrates attempts to discern any significant morphological structure in the surrounding galaxies and therefore any of the usual signs of interaction, it is gratifying to find, in the morphology of extended emission-line regions, a basis for identifying certain classes of interactions that puts the luminosity of the nucleus to good use.

We suspect, although we cannot yet demonstrate, that objects such as 4C37.43^{9,15}, which show extended emission with a more complicated morphology and velocity structure than 3CR249.1 and Ton202 (A.S. and J.W.M., in preparation), are simply

examples of interactions for which the interaction parameters and viewing angle make the interpretation more difficult. Other evidence for interactions involving luminous QSOs includes the presence of very close compact companions³, and a large-scale asymmetry in the stellar distribution surrounding the QSO Mkn1014 (J.W.M. and A.S., in preparation). We emphasize that only a minority of interactions are likely to leave traces easily visible at $z > 0.2$, and these examples probably represent only the most visible extreme of a larger population of interactions involving a sizable fraction of all high-luminosity QSOs. Thus, we believe that 3CR249.1 and Ton202 support the contention that interactions are important in making available the mass flow into the centre required to fuel luminous QSOs near the present epoch. We stress these qualifying terms: low-luminosity forms of nuclear activity may be sustainable by internal relaxation processes alone in a galaxy; certainly there are some

Seyfert galaxies that appear undisturbed by external influences. On the other hand, at early epochs, while interactions were no doubt more frequent, relatively large mass-flow rates into the centre might also have been available both from material on plunging orbits that had not yet been depleted and from late-arriving debris of galaxy formation processes.

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Cell type switching by DNA transposition in fission yeast

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The mating-type genes of Schizosaccharomyces pombe have been isolated. Restriction and heteroduplex mapping have demonstrated that cell type switching occurs by copy transposition of a 1.1 kilobase sequence from one of two silent storage cassettes to an expression locus at mat1. The switching event is initiated by a long-lived double-strand DNA break at mat1. The properties of this cut are discussed in relation to the known pattern of cell type switching.

SCHIZOSACCHAROMYCES pombe is a haploid yeast which grows by equational cell division in a nutritionally adequate medium but upon starvation undergoes conjugation between pairs of cells to form a temporary diploid zygote¹. Conjugation does not take place at random but is restricted to cells of the opposite mating type h^+ or h^- (ref. 2). In heterothallic strains cellular mating type is stably inherited through mitotic division but in wild-type (h^{90}) strains the mating type of the cell switches between h^+ and h^- with a frequency of approximately 30% per cell division^{3,4}.

Once conjugation has occurred, the diploid cell, containing both h^+ and h^- mating-type information, directly enters premeiotic S phase and after two meiotic divisions forms an ascus of four haploid spores. If, however, the newly-formed zygote is supplied with fresh nutrients just before initiation of meiosis, the diploid cell can reenter the mitotic cycle. Mating-type switching continues in dividing diploids to generate h^+/h^+ , h^-/h^- and h^+/h^- cells. Diploids homozygous at the mating-type locus are capable of further conjugation if starved, whereas h^+/h^- cells can only sporulate¹.

Genetic analysis of the mechanism of mating-type determination, initiated by Leupold^{2,5}, identified two elements, *mat1* and *mat2*, linked within 1.0 centimorgan on the long arm of chromosome II. One model⁶ proposed to explain mating-type switching suggested that a central 'flip-flop' promoter altered its orienta-

tion to activate genes resident at either *mat1* or *mat2*. However, it has now been shown⁷ that the mating-type region contains a third component, *mat3*, identified as the site of a mutation of minus information (*mat3B406*)⁸⁻¹⁰. Although *mat2* and *mat3* have never been separated by meiotic recombination (<0.001 centimorgan)^{7,11}, it has been proposed⁷ that *mat2* and *mat3* are two separate loci carrying silent stores of plus and minus mating-type information which become activated by their individual transposition to *mat1*. The resident allele at *mat1* would thus determine the momentary phenotype of the cell until replaced by a subsequent transposition event. Genetic evidence supporting this transposition-activation model has recently been obtained for both plus information (*mat2B102* mutation) transposing from *mat2* to *mat1*⁷ and minus information (*mat3B406* mutation) transposing from *mat3* to *mat1* (ref. 11).

This view of the mechanism of mating-type switching bears strong analogy to the 'cassette' model^{12,13} which proposed that in *Saccharomyces cerevisiae* mating-type switching involves the transposition of silent storage sites (*HML* and *HMR*) to an expression locus (*MAT*). The basic tenets of the 'cassette' hypothesis have been directly verified in *S. cerevisiae* by cloning and physical characterization of the mating-type genes¹⁴⁻¹⁶.

In this work a plasmid containing *mat-P* (h^+) activity, isolated previously by complementation in *Schiz. pombe*¹⁷, has been

used as a hybridization probe to isolate each component of the mating-type region. Restriction and heteroduplex analysis has revealed that mating-type switching in this yeast does indeed occur by copy transposition of separate cassettes to the *mat1* expression locus. In addition, the genetically identified *cis*-acting *smt* locus known to be involved in switching⁹ is shown to be the site of a relatively stable chromosomal double-stranded break at *mat1*. A hypothesis to account for the properties of this cut in relation to the known pattern of cell switching is presented.

Isolation of mating-type genes

The plasmid pMT_{3.2} containing *mat-P* activity and capable of integration at the mating-type locus was previously isolated from a gene bank of *Schiz. pombe* DNA contained in a yeast/bacterial shuttle vector¹⁷. Subcloning of pMT_{3.2} demonstrated that *Schiz. pombe* DNA in the plasmid contained a unique *Bam*HI site which cannot be disrupted without loss of *mat-P* function (unpublished data). Hybridization of a subclone of pMT_{3.2} containing the *Bam*HI site, to the *Schiz. pombe* genome, demonstrated that it contained repeated DNA. Since these hybridizing sequences were likely to contain mating-type information, the plasmid was used as a hybridization probe to recover further fragments from a λ (L47.1) bank prepared from *Hind*III digested *h*⁹⁰ (968) *Schiz. pombe* DNA. This resulted in isolation of four *Hind*III fragments, each of which was subcloned into both pTR262¹⁸, a derivative of pBR322 allowing positive selection for *Hind*III inserts and pDB262 (A. Wright, D.H.B. and P. M. Nurse, manuscript in preparation), a yeast/bacterial shuttle vector.

Of the *Hind*III fragments, two were of identical length (10.4 kilobases (kb)) while the others were 6.3 kb and 4.2 kb (Fig. 1). Restriction mapping revealed that the two 10.4 kb fragments contained many sites in common except that one contained a unique *Bam*HI site whereas the other lacked this site but contained instead a nearby *Eco*RI site. The exact relationship between each *Hind*III fragment was explored further by heteroduplex mapping (see below).

Each *Hind*III fragment contained in pDB262 was transformed into two yeast strains *leu1.32mat2B102* and *leu1.32mat3B406*, both capable of conjugation but defective respectively for plus and minus sporulation functions⁹. *Leu*⁺ transformants (see refs 19, 20 for details of transformation procedure) were grown up for a week on sorbitol containing plates after which the amount of sporulation was assessed. Transformants of pDB262 alone had no spores whereas those of the *Bam*HI containing 10.4 kb fragment gave approximately 20% sporulation in *mat2B102* and the other 10.4 kb fragment gave a similar frequency in *mat3B406*. No sporulation was observed for either fragment in the other strain. The 6.3 kb fragment gave very low levels of sporulation (~0.1%) in *mat2B102* but none in *mat2B406*, whereas the 4.2 kb fragment gave a similarly low percentage of spores in *mat3B406* but none in *mat2B102*. The plasmids contain either expressed or silent

copies of plus or minus *mat* information. The two alternative forms of the 10.4 kb expression fragment are hereafter referred to as *mat1-P* or *mat1-M* and the silent copies *mat2-P* (6.3 kb) and *mat3-M* (4.2 kb). [Formal verification of the colinearity of the genetic map of the mating-type locus and the restriction map in Fig. 1 has been established by use of strains carrying spontaneous deletions within the locus and will be published elsewhere (D.H.B. and A. J. S. Klar, manuscript in preparation).]

Mating-type information in 'cassettes'

Restriction mapping of the 10.4 kb *mat1-P* and *mat1-M* fragments revealed that they share common sequence for most of their length and hybridization of the *mat1-M* fragment to *Hind*III digested *h*⁹⁰ DNA shows that it has homology to each of the mating-type fragments (Fig. 4A). To investigate the spatial relationships between regions of homology the four *Hind*III fragments, contained in pTR262, were hybridized in each of six pairwise combinations and visualized by electron microscopy (Fig. 2). Before hybridization each molecule was digested with *Pst*I which cuts uniquely in the vector alone. In each combination except *mat1-P/mat1-M*, the vector sequences are seen as a long and a short double-stranded region at the ends of the hybrids. Contour lengths of the double- and single-stranded regions were determined from photographs of five molecules of each class and were used to generate the data summarized in Fig. 3.

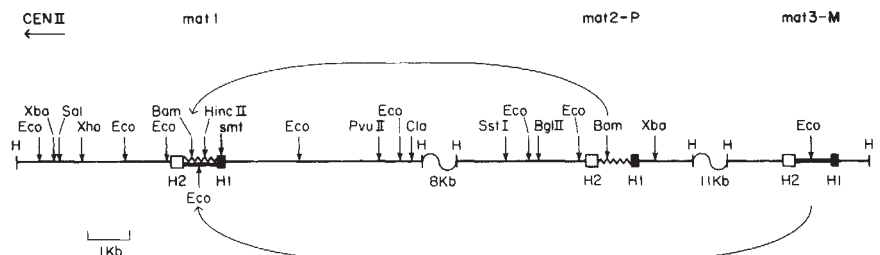
The heteroduplex analysis yielded the following conclusions. (1) *mat1-P* and *mat1-M* are homologous along their entire length except for a centrally located region of 1.1 kb (Fig. 2A). No significant difference could be found in the lengths of the P and M specific regions forming the bubble and they could not therefore be distinguished. (2) *mat1-P/mat2-P* and *mat1-M/mat3-M* each have a 1.4 kb region of homology which showed no significant difference in length between the two classes of hybrid (Fig. 2B, C). (3) *mat1-P/mat3-M*, *mat1-M/mat2-P* and *mat2-P/mat3-M* each have a common pattern of hybridization: a central single-stranded bubble of 1.1 kb is flanked on one side by a 190 base pair (bp) and on the other by a 90 bp region of double-stranded homology (Fig. 2D-F). These regions of homology, termed H₂ and H₁ (Fig. 1) are very short and it cannot be stated with certainty that there are no slight differences of length between each pairwise combination of molecules. The estimates of 190 bp and 90 bp were averaged from the three sets of heteroduplexes.

The restriction maps of *mat1-P* and *mat1-M* have been shown to differ by a *Bam*HI and *Eco*RI site unique to each molecule. Figure 3 shows the positions of these two sites. The two sites lie within the plus specific (P) and minus specific (M) regions of *mat1*. The unique *Bam*HI and *Eco*RI sites of *mat2-P* and *mat3-M* lie within the regions of *mat1-P/mat2-P* and *mat1-M/mat3-M* homology.

In summary, the heteroduplex results demonstrate that each of the *mat* loci consists of a 1.1 kb region of plus specific (P)

Fig. 1 Restriction map of four *Hind*III fragments containing mating-type genes. The 10.4 kb *mat1* fragment exists in two forms; the variable region is marked ~ for *mat1-P* and — for *mat1-M*. The boxes marked H₁ and H₂ are regions of homology common to each copy of *mat* and *smt* marks the position of a double-stranded cut generated *in vivo* (see text). The arrow marked CENII indicates that the centromere of chromosome II lies to the left of the *mat* region as drawn in the figure. The orientation of the *Hind*III fragments and distances between them are derived from other work (D.H.B. and A. J. S. Klar, in preparation). The restriction mapping is complete for the enzymes shown except for *Hinc*II; only the *Hinc*II site within *mat1-P* is displayed.

Methods: The *Hind*III fragments were isolated from a bank of *h*⁹⁰ (968) DNA constructed in the λ vector L47.1³⁸. Approximately 2×10^4 plaques were plated from a packaged ligation mix and lifted onto nitrocellulose filters³⁹. A *Bam*HI/*Hind*III subclone of pMT3.2¹⁷ was nick-translated and used to probe the λ plaques. Approximately 20 plaques hybridizing to varying intensity were purified and those containing 6.3 kb, 4.2 kb and two forms of 10.4 kb *Hind*III fragments were identified.



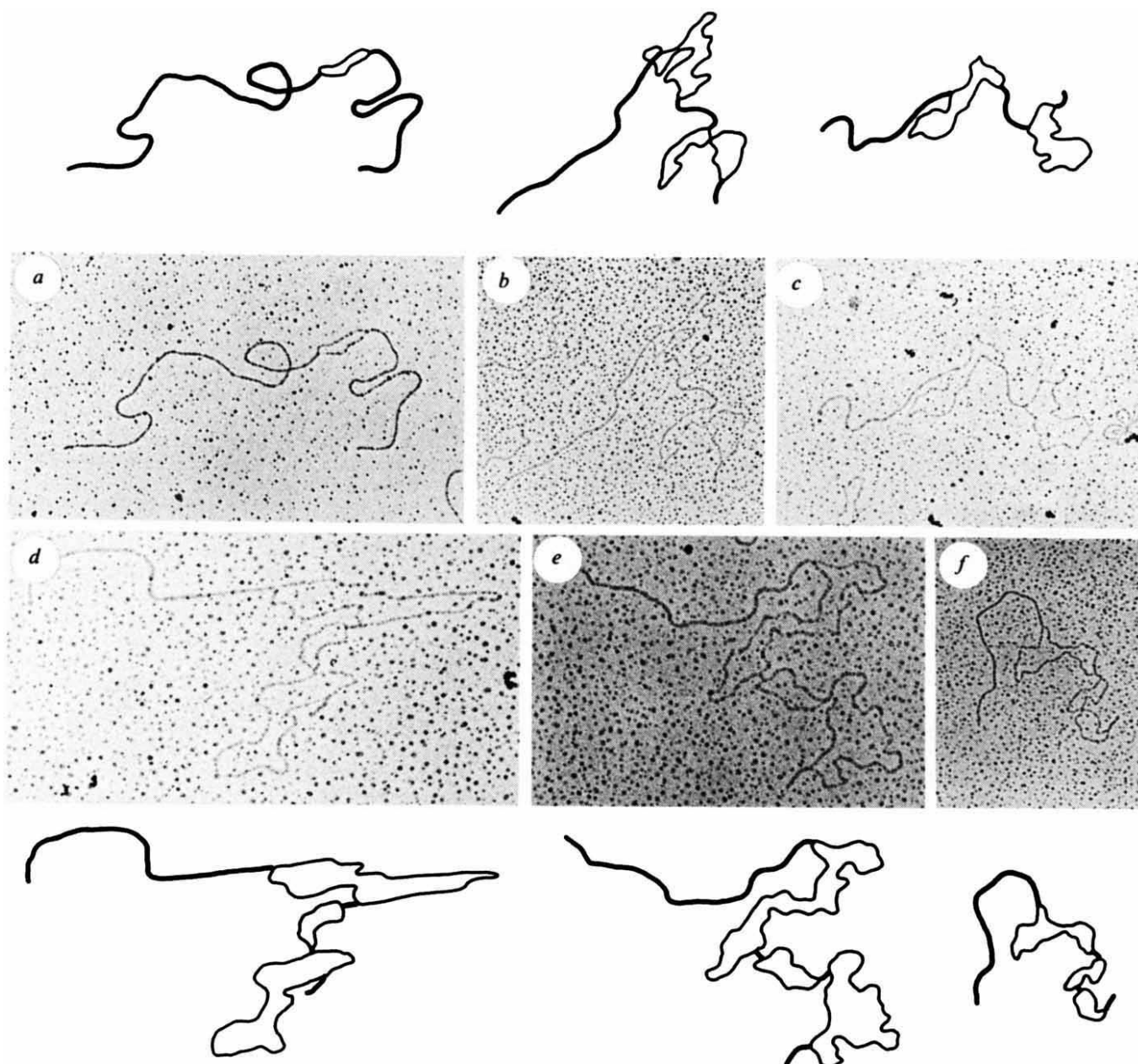


Fig. 2 Heteroduplex structures formed between plasmids containing *mat* *Hind*III fragments. A, *mat1-P/mat1-M*; B, *mat1-P/mat2-P*; C, *mat1-M/mat3-M*; D, *mat1-P/mat3-M*; E, *mat1-M/mat2-P*; F, *mat2-P/mat3-M*. Above or below each photograph is an interpretation of the heteroduplex structure showing double-stranded and single-stranded regions as heavy and light lines respectively. The long arm of annealed vector sequence is adjacent to the side of the insert closest to the centromere and is to the left of each photograph.

Methods: Each *Hind*III *mat* fragment was subcloned into the unique *Hind*III site of pTR262¹⁸ and plasmid was prepared from those isolates carrying the insert in the same orientation. 1 µg of each of the four plasmids was digested with *Pst*I (New England Biolabs) which cuts in the vector alone and hybridized in each of six pairwise combinations in 50% formamide. The hybrids were spread on a 10% formamide hypophase⁴⁰, taken up on parlodin coated grids and photographed on a Phillips 201 electron microscope. Contour lengths of regions of each hybrid were measured with a planimeter (Numonics Corporation).

or minus specific (M) sequence bounded proximally by a 190 bp (H_2) and distally by a 90 bp (H_1) region.

Switching by copy transposition

The heteroduplex and transformation experiments suggest that *mat1-P* and *mat1-M* are alternative states of a single genetic locus which can express either h^+ or h^- functions. The finding that *mat2-P*, the presumed silent plus cassette, contains a *Bam*HI site and *mat3-M*, the silent minus cassette, contains an *Eco*RI site, suggests that mating type switching involves transposition of at least 1.1 kb of DNA from either *mat2-P* or *mat3-M* to *mat1*, since *Bam*HI and *Eco*RI sites are found at equivalent locations in *mat1-P* and *mat1-M* respectively.

To test this hypothesis, use was made of a strain having a reduced rate of switching due to a mutation in a gene, *swi3*, which is unlinked to the mating-type locus (ref. 7 and R. Egel, personal communication). *swi3^{h90}* spores were obtained from a self-mating and each spore of the ascus was separated by conventional micromanipulation on a nutrient plate. The colonies developing from each spore were grown up in 1 ml liquid culture and DNA was prepared. Part of the colony was also taken for mating-type testing (see Fig. 4 legend).

Figure 4B shows a Southern transfer of a *Bam*HI/*Eco*RI double digest of the DNA of each spore of two tetrads hybridized with a *mat1-P* subclone, pEP6 (see Fig. 4 legend). This shows that the upper band (2.4 kb *Bam*HI/*Eco*RI fragment) is predominant in the h^+ cultures and the lower band (2.25 kb *Eco*RI

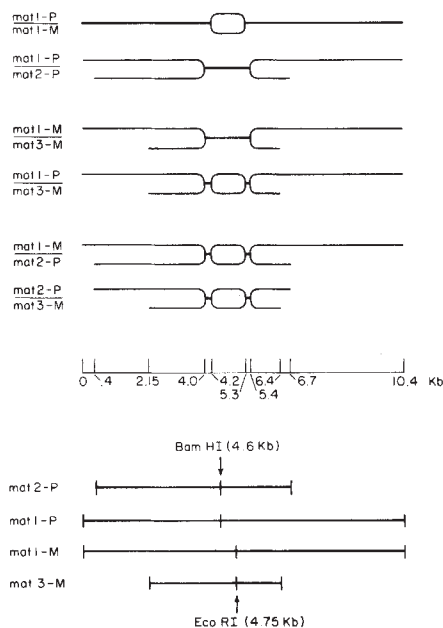


Fig. 3 Schematic diagram summarizing the results of heteroduplexing between four plasmids carrying *mat* genes. The vector is excluded from the figure. Lengths of double-stranded (heavy line) and single-stranded (light line) regions of each structure were measured on a planimeter and calibrated in kilobases by comparison with single and double-stranded full length pBR322 spread in the same mixture with the heteroduplexes. The lengths shown are derived from the average measurements of five examples of each heteroduplex structure and are drawn on a scale of 0–10.4 kb. Shown beneath the heteroduplexes are the *Hind*III inserts of each mating-type plasmid, showing the relative position of the *Bam*HI site unique to the *mat*-P cassettes and the *Eco*RI site contained 150 bp distally in the *mat*-M cassettes.

fragment) is stronger in h^- cultures. The difference of intensity segregates 2:2 in tetrads. Since *swi3* does not abolish switching but merely reduces it to approximately 2% per cell division (R. Egel, personal communication), considerable switching will have occurred as a single spore grows up in 1 ml culture ($\sim 10^8$ cells).

This accounts for the presence of both 2.4 and 2.25 kb bands in each DNA preparation. Note that these data do not reveal a difference in length between *mat1*-P and *mat1*-M but rather the existence of a *Bam*HI site at *mat1* in an h^+ cell and an *Eco*RI site at *mat1* in an h^- cell. It is concluded that a mating type switch involves copy-transposition of at least the 1.1 kb P or M regions of *mat2*-P or *mat3*-M to *mat1* with concomitant loss of the resident allele at *mat1*. The P region differs from M not only by a *Bam*HI site but also by *Hinc*II, *Bcl*I and *Mlu*I sites unique to P cassettes (*Bcl*I and *Mlu*I sites not shown in Fig. 1). This suggests that P and M contain quite different sequence and the fact that one plus specific transposable mating-type mutation (*mat2B102*) is opal suppressible²¹ indicates that the P region contains at least part of a structural gene. The extent to which the H_1 or H_2 regions are transposed during a switch has not been determined.

Switching initiation

Figure 4A shows that *mat1*-M hybridizes not only with bands corresponding to *mat1*-M, *mat1*-P, *mat2*-P and *mat3*-M, but also with two other bands, marked *smt*(P) and *smt*(D), which at 5.4 kb and 5.0 kb add up to the size of the 10.4 kb *mat1* *Hind*III fragment. This suggests that the two bands might be products of a double-stranded cut generated *in vivo* at *mat1*. To map the hypothesized cut, h^{90} (968) DNA was digested with *Hind*III alone or with *Hind*III and *Xho* and hybridized with a probe carrying sequences flanking but not containing the mating-type information of *mat1* (Fig. 5B). As expected this probe hybridizes with both the 5.4 and 5.0 kb bands and the blot shows that the 5.4 kb fragment contains an *Xho* site which divides the molecule into 1.7 and 3.7 kb fragments. These data are consistent with the existence of a double-stranded cut 5.4 kb distal to the left-hand *Hind*III site of *mat1*. The position of the cut was further mapped at 0.75 kb distal to the *Bam*HI site of *mat1*-P (data not shown). This places the cut site, marked as *smt* in Fig. 1, within or very close to the H_1 homology region. Since H_1 is so short (~ 90 bp) it has not been possible to define exactly where the cut lies with respect to H_1 , but it is found when either P or M information is at *mat1*. In *Sacch. cerevisiae* a cut is detectable within a few base pairs of the Y-Z boundary²² which is analogous to the PH_1 or MH_1 boundary described here.

Fig. 4 **A**, Autoradiograph of a Southern transfer⁴¹ of h^{90} (968) DNA digested with *Hind*III (lane 1) or *Hind*III and *Bam*HI (lane 2) hybridized with a nick-translated plasmid containing the full length 10.4 kb *mat1*-M *Hind*III fragment. The 10.4 kb *mat1*, 6.3 kb *mat2* and 4.2 kb *mat3* bands are shown in addition to *smt*(P) and *smt*(D), the proximal and distal fragments generated by the *in vivo* cut of *mat1* at *smt*. *Bam*HI/*Hind*III digestion of h^{90} DNA reduces the intensity of the *mat1* 10.4 kb *Hind*III fragment indicating that it contains two molecular types. The 10.4 kb fraction cut by *Bam*HI is reduced to 5.8 kb and 4.6 kb. *mat3* is uncut by *Bam*HI but *mat2* is cut to yield a 4.2 kb band (observed by *mat3*) and a 2.1 kb band (run off this gel). **B**, Autoradiograph of a Southern transfer of h^{90} *swi3* DNA digested with *Eco*RI and *Bam*HI and hybridized with pEP6, which contains the distal 2.4 kb *Eco*RI/*Bam*HI fragment derived from *mat1*-P (Fig. 1).

Methods: Each lane contains the DNA of a single spore from two complete tetrads grown as a colony and then inoculated into a 1 ml culture of YEA (0.5% yeast extract, 3% glucose, 75 mg l⁻¹ adenine). The predominant mating-type present in the colony was assessed by patching part of the colony onto a minimal agar plate with an h^- and h^{+N} tester strain patched close on either side. Each colony generated a line of cells staining with iodine vapour, a spore-specific stain, with one tester and a barely detectable line with the other. This allowed assignment of the predominant mating type of the colony. The predominant mating type is marked + (h^+) or - (h^-) in each lane. Chromosomal DNA was prepared as described previously except that zymolyase 6000 replaced Novo SP234²⁰.

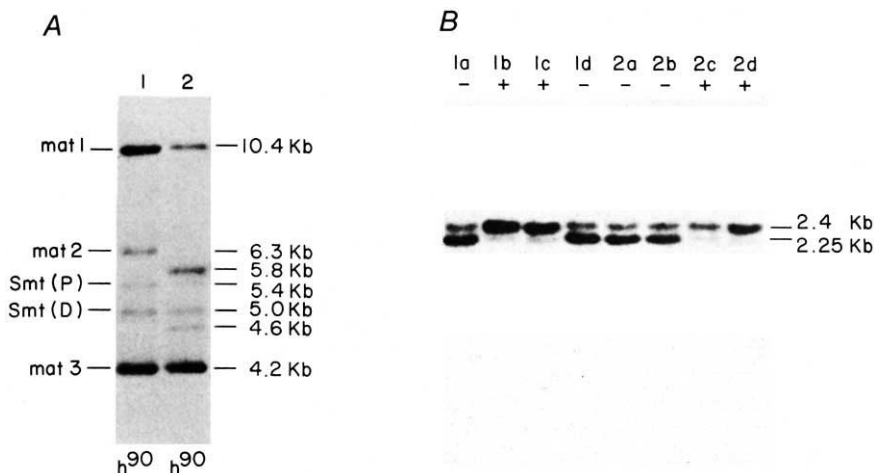
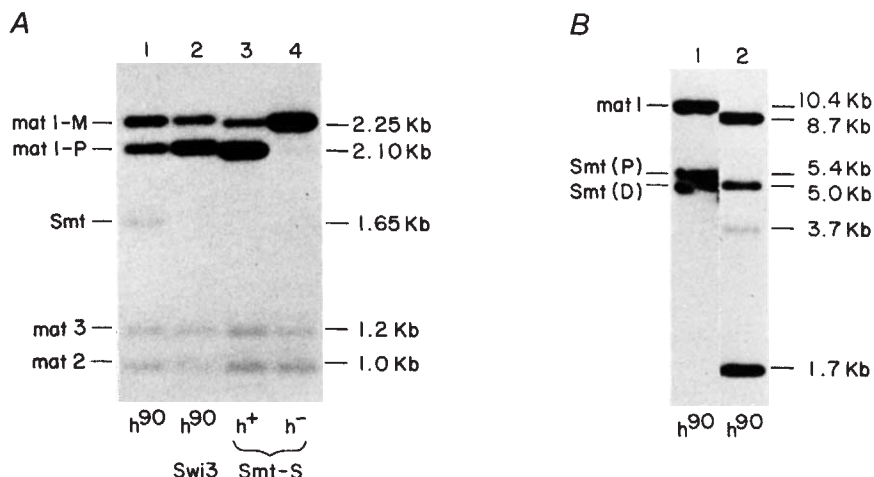


Fig. 5 A. Autoradiograph of a Southern transfer of h^{90} (968), h^{90} *swi3* and h^{90} *smt-s* DNA digested with *EcoRI* and *HincII* and hybridized with nick-translated plasmid pEP6, containing the *BamHI/EcoRI* fragment described in the legend to Fig. 4B. The position of the *mat1-M* 2.25 kb *EcoRI* fragment, the *mat1-P* 2.1 kb *HincII/EcoRI*, and 1.65 kb *smt/EcoRI* bands are shown. The identity of the 1.2 kb and 1.0 kb bands as derivatives of *mat3* and *mat2* was established from *HincII* mapping of plasmids containing these sequences. The mating type of *smt-s* h^{+} and *smt-s* h^{-} colonies was determined by the method described in the legend to Fig. 4. **B.** Autoradiograph of a Southern transfer of h^{90} (968) DNA digested with *HindIII* (lane 1) or *HindIII* and *Xho* (lane 2) hybridized with nick-translated plasmid containing two *EcoRI* fragments, one from either side of the central region of *mat1-P*. The probe hybridizes only to *mat1* or derivatives of it.



If the double-stranded cut at *mat1* is an intermediate in switching it might be altered in amount in switching defective strains. Switching is reduced by three classes of mutation: those unlinked to the mating-type locus (*swi*)²³, those arising at high frequency by DNA rearrangement of the *mat* locus (refs 17, 24 and D.H.B. and A. J. S. Klar, in preparation) and finally by mutations at *mat1* which neither arise nor revert at high frequency^{9,25}. The third class of mutations is *cis*-acting and has been used to define a locus (*smt* for switching of mating-type), required for efficient switching, which maps approximately 0.1 centimorgan distal to *mat1* mutations⁷. *smt* mutations are defined not only by reduced switching, an event which only occurs in *cis*, but also by a reduction in the extremely high rate (2% per cell division) of mitotic cross-over which is observed between *mat1* and *mat2* in switching diploids^{9,26}.

To investigate the double-stranded cut in switching defective strains use has been made of a *swi3* mutation and also of *smt-s* C13-11, which reduces h^{+} to h^{-} switching to 10^{-2} per cell division and h^{-} to h^{+} switching to 10^{-4} per cell division⁹. Predominantly h^{+} or h^{-} colonies were grown to saturation in 20 ml cultures for preparation of DNA. Figure 5A shows the pattern of hybridization obtained using the *mat1* subclone pEP6 as probe against *EcoRI/HincII* double digests of the yeast DNA. h^{90} DNA (lane 1) has the 2.1 kb *EcoRI/HincII* *mat1-P* band and the 2.25 kb *EcoRI* *mat1-M* band in equal amount and has a 1.65 kb band representing the *smt/EcoRI* fragment distal to *mat1*. The intensity of this band is very much reduced in both the *swi3* h^{90} and *smt-s* h^{90} strains (lanes 2-4). *smt-s* h^{-} DNA lacks the 2.1 kb *mat1-P* band since the strain switches so infrequently to h^{+} ($\sim 10^{-4}$). The position of the *mat1-P* and *mat1-M* bands is slightly displaced in *smt-s* DNA, indicating a deletion of approximately 50 bp in a region common to both alleles of *mat1*. Heavy overexposure of the autoradiograph in Fig. 5A showed the weak presence of the 1.65 kb band in *swi3* and *smt-s* DNA. In the *smt-s* strain this band is also slightly displaced, suggesting that the strain carries a deletion distal to the *smt* cut site. This result is consistent with the finding that *smt-s* C13-11 maps 0.1 centimorgan distal to *mat1*⁹ and although the mutation interferes with switching it is not healed by a switch. It must therefore lie outside the region of *mat1* which is displaced during a switch.

These data strongly suggest that the genetically defined *smt* locus is the site of a double-stranded DNA cut which has a role in mating-type switching. A similar conclusion has been drawn about the cut detected at the Y-Z boundary in switching strains of *Sacch. cerevisiae*²². In both yeasts the formation of a double-stranded break is probably a step in the initiation of a unidirectional gene conversion event, during the resolution of which it gets healed. In fission yeast the double-stranded *smt* cut also appears to act as an extremely active hot-spot for mitotic and meiotic recombination in diploids^{9,26}.

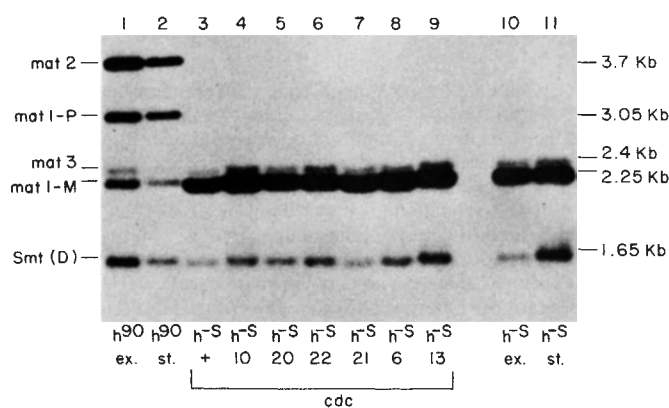


Fig. 6 Autoradiograph of a Southern transfer of DNA digested with *EcoRI* and hybridized with pEP6, an *EcoRI/BamHI* subclone of *mat1-P* as described in the legend to Fig. 4B. The lanes contain DNA from the following strains: 1, h^{90} (968) during exponential growth (5×10^7 cells per ml in minimal medium⁴² as modified in ref. 43). 2, h^{90} (968) after 4 h of exposure to minimal medium lacking a nitrogen source (NH_4Cl). St. signifies that the cells were in stationary phase rather than in exponential growth (ex) by this time. 3, DNA from h^{-s} (972) during exponential growth in yeast extract medium at 36°C . 4-9, DNA from the same strain but for a single temperature-sensitive *cdc* (*cdc10.129*, *cdc20M18*, *cdc22M45*, *cdc21M68*, *cdc6.23*, *cdc13.117*) mutation in each. The cells were collected 4 h after shift-up to the restrictive temperature (36°C). 10 and 11, h^{-s} (972) grown to mid-log phase in minimal medium or after 16 h exposure to the same medium lacking a nitrogen source (NH_4Cl). The h^{-s} (972) strain contains a fusion of the *mat2* and *mat3* cassettes retaining M mating-type information (D.H.B. and A. J. S. Klar, in preparation). It thus contains the 2.4 kb distal *mat3* *EcoRI* fragment but lacks the *mat2* and *mat1-P* fragments. The product of the *in vivo* cut, *Smt(D)*, is thus generated exclusively from *mat1-M*.

Double-stranded cut inheritable?

Although expression of all sexual functions in fission yeast is limited to conditions of nutritional limitation²⁷, mating-type switching does occur during mitotic growth of both haploids and diploids^{3,4}. It is therefore of interest to investigate the physiological conditions and stage of the cell cycle in which the double-stranded cut is formed and healed.

Strains carrying one of six temperature-sensitive *cdc* mutations²⁸ were constructed with the h^{-s} mating type. h^{-s} strains have normal *mat1-M* functions but the minus mating type is stable as a result of the fusion of the *mat2* and *mat3* cassettes with loss of P information²⁹. In the first experiment (Fig. 6, lanes 3-9) each *cdc* strain was shifted to the restrictive tem-

perature (36 °C) during exponential growth to induce cell cycle arrest. DNA was prepared 4 h after the temperature shift during which time considerable cell elongation had occurred except in the *cdc*⁺ strain (lane 3). In a second experiment DNA was isolated from *h*^{-s} and *h*⁹⁰ cultures either during exponential growth (Fig. 6, lanes 1, 10) or after 4 h (*h*⁹⁰, lane 2) or 16 h (*h*^{-s}, lane 11) of nitrogen starvation. Nitrogen source starvation arrests cells in G₁ and in these conditions mating is initiated within 4 h in *h*⁹⁰ strains²⁷ whereas *h*^{-s} merely becomes quiescent.

The data in Fig. 6 show that, allowing for small differences in the DNA loading of each track, the amount of double-stranded *smt* cut (~20% of *mat1*) is not markedly different in any of these varying conditions. This result is surprising particularly in the case of nitrogen-starved cells since in this condition growth and division rapidly cease and it is most unlikely that switching continues. It appears that in the absence of division the cut is stabilized, presumably either by binding of protein, by base-pairing of a long 'sticky-end' of a staggered cut or possibly by heteroduplexing with the H₁ region of *mat2-P* or *mat3-M*.

The *cdc* mutants used in these experiments arrest cells at different stages of the cycle (*cdc10*, *cdc20* and *cdc22* in G₁, *cdc21* in S phase, *cdc6* in G₂ and *cdc13* in mitosis)^{30,31}. The *cdc13* result is particularly striking since the division cycle becomes arrested by this mutation just before mitotic separation of the chromosomes which can be seen as three discrete staining bodies³¹. Cells arrested at the *cdc13* block point and also at that of *cdc10*, the earliest known gene function required after nuclear division³¹, each contain similar amounts of double-stranded *smt* cut. It thus seems highly probable that the cut is not only stable in the absence of cell proliferation but can also be inherited through mitotic division.

Two hypotheses can account for these data. The cut could form totally at random with respect to cell cycle events and after some time could be healed by a switching event. Alternatively, generation of the cut could be regulated very strictly in the cell cycle if it is assumed that healing of the cut is likewise regulated and occurs at almost exactly the same stage of the cycle but after a delay of one cell division. Arrest at no stage of the cycle would therefore cause enhancement or depletion of the amount of cut.

Study of the pattern of switching gives information distinguishing between these alternatives. Miyata and Miyata⁴ analysed switching by observing the distribution of zygotes formed in the 'four-lined' cell resulting from the two terminal cell divisions which occur after abrupt removal of a nitrogen source. It was found that sister cell matings predominated over cousin cell matings (8:2) and that a 'four-lined' cell never contained two zygotes. It was concluded that the potential to switch segregates asymmetrically at division such that when one cell gives rise to a mixed pair of daughters its sister cell never does so. This asymmetry did not segregate according to a mother-daughter rule^{32,33} but was random with respect to new (1 division scar) and old (>1 division scar) cells within the four-lined cell. Egel¹¹ has shown that the asymmetry of switching potential is equally apparent in switching diploids and is inherited independently in each set of chromosomes. This suggests that the asymmetry arises from a property inherent to the chromosome itself rather than from the nature of the cytoplasm into which chromosomal segregation occurs.

The predominance of sister cell matings and the apparent lack of paired cell switching¹¹ implies that switching does not occur at random with respect to the cell cycle but must take place during or after replication of *mat1*. The following hypothesis is proposed to account for the paradox generated by the apparent lack of regulation of the *smt* cut, the predominance of sister cell mating and the asymmetric distribution of switching potential. At the time of replication of *mat1*, some property of *smt* is inherited by one chromatid but not the other. This leads to one of the two chromatids being cut at *smt* by a continuously present site-specific endonuclease. The cut is stabilized and

inherited by one sister cell at division. It remains until the following S phase during which it is healed by a mating-type switch. Since only one daughter of the subsequent division will have switched, one strand of the *smt* cut duplex must be healed either without switching or by undergoing a homologous switch. In this model both *smt* cutting and switching are presumed to occur during S phase but one complete cycle, during which the cut is stabilized, intervenes between the two events. Furthermore, only one strand of the double-stranded cut is used in the switch.

The pattern of mating-type switching in fission yeast is quite different from that in *Sacch. cerevisiae* in which cells always switch in pairs^{32,33}. There is an asymmetry of switching potential in budding yeast but unlike the situation in fission yeast it is reflected in a restriction of switching to old cells (mothers). Daughter cells (buds) never switch until they have become mothers^{32,33}. Recently an endonuclease specifically recognizing the Y-Z junction of *MAT* has been found (R. Kostriken *et al.*, in preparation) and this endonuclease is apparently not produced by buds³⁴. Discovery of a site-specific endonuclease recognizing the *smt* site of *mat1* would contribute to understanding the molecular basis of the pattern of cell type switching in fission yeast. Such an endonuclease might be coded for by one of the 10 *swi* genes already identified (ref. 23 and R. Egel, personal communication).

Conclusion

Despite the evolutionary divergence between *Sacch. cerevisiae* and *Schiz. pombe*^{35,36}, there are clear parallels in the organization of their mating-type genes. In both yeasts, mating-type switching occurs by copy transposition of silent 'cassettes' into an expression locus and in each the recombination event of switching is initiated by a double-stranded cut at the recipient locus. The rules governing the generation of the cut may however be quite different. Formation of a double-stranded break has been proposed as a general initiating step in meiotic cross-over and gene conversion events in fungi³⁷. Mating-type switching provides the only examples of normal recombination events which have been shown to occur by this mechanism.

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LETTERS TO NATURE

Fast pulsations in the solar corona

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Pulsations in radio emission from the solar coronal plasma have been detected for over a decade¹⁻⁹. The oscillations are quasi-periodic, with periods of typically a second or so. Recently, sub-second time structures have been found in hard X rays¹⁰, and simultaneously in hard X rays and microwaves¹¹. Here we examine whether magnetohydrodynamical oscillations in a density enhancement, treated for simplicity as a straight magnetic slab, can explain the observed short periods. A dense region in the corona (for example, a loop) can act as a wave trap, and symmetrical oscillations within that trap must be of short wavelength with correspondingly short period. An impulsive source (such as a flare) naturally gives rise to a quasi-periodic disturbance. Such oscillations are closely akin to the Pekeris modes of oceanography, the Love waves of seismology and the dielectric waves of fibre optics.

One of the major puzzles presented by the observations of quasi-periodic pulsations in the solar corona is why the periods of the oscillations are so low, being much lower than typical source sizes and wave speeds would suggest. As Takakura *et al.*¹¹ have illustrated, an observed source size of 1.5×10^4 km and a supposed Alfvén speed of 3×10^3 km s⁻¹ yields a characteristic time of 5 s, which is an order of magnitude longer than the observed 0.3 s period. Evidently, unresolved small-scale inhomogeneities within the source region are determining the characteristic periods of pulsations.

We point out here a possible explanation for such short periodicities. Our theoretical explanation is based on the magnetohydrodynamical behaviour of a fast magnetoacoustic oscillation in a low- β (coronal) atmosphere. The solar corona is highly structured; coronal loops and magnetic arches are evident in X-ray and extreme UV photographs (see refs 12, 13). In a structured atmosphere, with density inhomogeneities present, the fast wave is trapped in regions of high density (low Alfvén speed), such regions acting as waveguides. Moreover, a fast magnetoacoustic wave in a dense loop possesses propagation cut-off, admitting free (nonradiative) symmetrical oscillations only for sufficiently short wavelengths¹⁴. It is this reduced wavelength, we claim, that gives rise to the observed low periods.

Magnetohydrodynamical pulsations of a loop have in fact been invoked previously as a possible explanation of low periodicities^{2,15}, but earlier treatments have overlooked the guided modes of oscillation exploited here.

The above described features of fast oscillations in a coronal loop may be most conveniently illustrated by examining the magnetoacoustic waves in the extreme of zero sound speed. Transverse oscillations $v_\perp$ are then described by the wave equation

$$\frac{\partial^2}{\partial t^2} v_\perp = v_A^2 \nabla^2 v_\perp \quad (1)$$

where v_A is the Alfvén speed in a constant (and straight) magnetic field, and ∇^2 is the two-dimensional laplacian. If the gas density varies in a direction perpendicular to this field, as is appropriate for the corona, then the Alfvén speed squared will vary inversely with the density, being lower where the density is higher.

Equation (1) may be readily solved for a 'coronal loop', modelled as a density inhomogeneity of width $2a$ in a uniform and straight field (the magnetic slab). The wave equation then yields the dispersion relations¹⁴

$$\tan \left(\frac{\omega^2}{v_A^2} - k^2 \right)^{1/2} a = \left(\frac{k^2 - \frac{\omega^2}{v_{Ae}^2}}{\frac{\omega^2}{v_A^2} - k^2} \right)^{1/2} \quad (2)$$

for kink modes, and

$$\tan \left(\frac{\omega^2}{v_A^2} - k^2 \right)^{1/2} a = - \left(\frac{\frac{\omega^2}{v_A^2} - k^2}{k^2 - \frac{\omega^2}{v_{Ae}^2}} \right)^{1/2} \quad (3)$$

for the sausage modes. In the above, ω is the frequency of the wave, k is the wavenumber along the field, and v_A and v_{Ae} are the Alfvén speeds inside and outside the slab inhomogeneity, where the gas densities are ρ_0 and ρ_e , respectively. (The condition that disturbances decline exponentially to zero away from the slab has been imposed in deriving equations (2) and (3).)

It is a pleasing coincidence that relations equivalent to equations (2) and (3), which here describe the propagation of magnetic waves trapped in a coronal inhomogeneity, arise in seismology and oceanography¹⁴. In particular, equation (2) is exactly equivalent to Love's relation^{16,17} describing trapped waves in the Earth's mantle, and equation (3) is equivalent to the relation studied by Pekeris¹⁸ that governs propagation in an ocean layer^{17,19}. Furthermore, relations analogous to equations (2) and (3) arise in fibre optics^{20,21}, although the analogy between the various fields appears to have gone unnoticed.

Two important conclusions can be drawn from equations (2) and (3). First, solutions (there are an infinity of them) with real ω and k exist only if $v_{Ae} > v_A$, and then $kv_A < \omega < kv_{Ae}$. Thus, it is regions of density enhancement that support trapped oscillations. Second, inspection of equation (3) shows that the symmetrical oscillations can only occur if k is sufficiently large, namely if

$$k > k^{\text{crit}} \equiv \frac{\pi}{2a} \left(\frac{v_A^2}{v_{Ae}^2 - v_A^2} \right)^{1/2}$$

(By contrast, the kink mode occurs for all k .) This cut-off in the symmetric oscillations implies that only short-wavelength modes can occur as trapped modes.

The period $\tau (= 2\pi/\omega)$ of symmetrical oscillations can be readily estimated at their onset. Symmetrical oscillations first occur when $\omega = kv_{Ae}$ and $k = k^{\text{crit}}$, and so have a period

$$\tau = \frac{4a}{v_A} \left(1 - \frac{\rho_e}{\rho_0} \right)^{1/2} \quad (4)$$

on noting that in the undisturbed state magnetic pressure balance implies that $\rho_0 v_A^2 = \rho_e v_{Ae}^2$. Thus, if the gas within a coronal loop is much denser than that in the magnetic environment, so that $\rho_0 \gg \rho_e$, then equation (4) reduces to $\tau = 4a/v_A$. For example, taking $v_A = 3,000 \text{ km s}^{-1}$, we obtain $\tau = 1 \text{ s}$ ($\tau = 0.3 \text{ s}$) for a magnetic slab of width $2a = 1,500 \text{ km}$ ($2a = 450 \text{ km}$).

There are two applications of the above, depending on whether the oscillations are standing waves or propagating waves. If the oscillations are standing waves in a closed structure, such as a coronal loop with its footpoints anchored in the dense photosphere–chromosphere, oscillations within the loops have wavelengths that are defined by the length of the loop. Thus, in a loop of length L we may set $k = n\pi/L$, for integer $n = 1, 2, 3, \dots$. Only those oscillations with sufficiently large n , and thus sufficiently small wavelength, will result in a normal mode (satisfying $k > k^{\text{crit}}$). For example, an emission region of extent $L = 1.5 \times 10^4 \text{ km}$ (as quoted by Takakura *et al.*¹¹) and an assumed external density of $\rho_e = \frac{1}{10}\rho_0$ can support a pulsation of period 0.3 s^{-1} if there are about 12 nodes in the oscillation, the nodes being distributed along the length of the emission region (loop); a smaller ρ_e gives fewer nodes.

Symmetrical oscillations in a loop locally modulate the magnetic field. If this occurs at the mirror point of a trapped particle, this point moves up or down in altitude. It is thus periodically located at places of low and high density. Most of the bremsstrahlung (hard X-ray) emission occurs near the mirror points of the particle's orbit. The varying density at this point then modulates the X-ray flux and leads to the observed oscillation. The associated microwave radiation is generally assumed to originate by the synchrotron mechanism from a fraction of the loop (say at the top). A local fluctuation of the magnetic field modulates the emitted flux proportional to the square of the field strength. Observations¹¹ then require oscillations of the field with an amplitude of $\sim 0.5\%$. During a compression the microwave flux is enhanced, but the mirror points move into the regions of lower density, thus reducing the X-ray emission. Such an anticorrelation has indeed been observed¹¹.

Turning now to a discussion of propagating (in longitude) waves, as opposed to standing modes, we note that for an impulsively generated disturbance all possible wavenumbers k contribute to the evolution of the wave. Suppose a disturbance is initiated, presumably by a flare, at some low level in the atmosphere. This source may be at a footpoint of a loop or at the base of an open field region. Then, at a height h above that level, the resulting disturbance will be a Fourier integral over k , with ω given by equations (2) or (3). Fortunately, such Fourier integrals have been analysed by Pekeris¹⁸ for oceanic disturbances, and his results can be applied to the present coronal problem, with only minor changes. Figure 1 sketches the temporal behaviour at a point distanced h km from the impulsive source. The disturbance comprises several phases, beginning with a periodic phase (as low-frequency components arrive at the observation point), followed by a quasi-periodic phase (as both high and low frequencies arrive and interact), and finally a decay phase (as the signal passes). The quasi-periodic phase is generally much stronger in amplitude, lower in 'periodicity', than the earlier periodic phase. Note also that the decay phase in Fig. 1 is an adiabatic process and not an indication of dissipative effects.

As a numerical illustration of Fig. 1, suppose that $v_A = 2,000 \text{ km s}^{-1}$ and $v_{Ae} = 4,000 \text{ km s}^{-1}$ (so that $\rho_0 = 4\rho_e$). Then, observing in the density enhancement at a height $h = 10^5 \text{ km}$ (say) above a flare, one would detect the onset of a periodic oscillation (with period given by equation (4)) 25 s after the initiation of the flare. This periodic phase lasts for about 25 s, when the quasi-periodic phase begins, lasting for 7 s (for a calculated minimum group velocity of $1,760 \text{ km s}^{-1}$) before the decay phase occurs. Thus, in this illustration, the quasi-periodic phase begins some 50 s after the onset of the flare and 'switches off' 7 s later.

How does the theoretical prediction of Fig. 1 compare with the available observations? To answer this, it should be borne

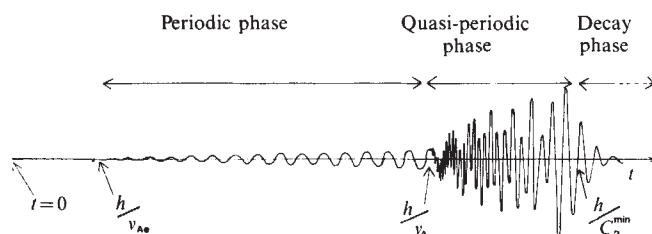


Fig. 1 A sketch of the evolution of an impulsively generated sausage mode (after Pekeris¹⁸; see also refs 17, 19). The disturbance is supposed to be initiated by a flare in the vicinity of the base of the active region, giving rise to a fast wave propagating in a density enhancement. The profile is the theoretically predicted behaviour at a height h in the density enhancement. Note the various phases in the oscillation: the onset of a periodic mode, followed by a stronger quasi-periodic phase, and finally an amplitude decay (Airy) phase (at constant frequency).

in mind that Fig. 1 pertains to the ideal case of a symmetrical disturbance generated by a single impulse. In practice, we may expect that asymmetrical (kink) oscillations are also generated by flares and the flare itself may consist of a sequence of impulsive events rather than a single impulse. Taken together, these effects will, in some events at least, considerably complicate the simple picture presented here. We may anticipate that an observational record will exhibit modulation of the symmetrical oscillations (by the kink waves), and a number of quasi-periodic phases may be present (reflecting the number of impulsive sources provided by the flare site). Furthermore, even if a symmetrical oscillation is generated by a single impulse, the lower amplitudes exhibited in the periodic phase (immediately before the onset of the quasi-periodic phase) may well pass unnoticed in the background noise, so that only the stronger amplitude quasi-periodic phase is apparent. Only a careful search of the evolution record might reveal the presence of the earlier periodic phase.

Despite the above qualifications the agreement between observations and theory is encouraging. In particular, the sudden onset of a quasi-periodic phase followed eventually by a decay phase is strikingly similar to the record presented by McLean *et al.*⁴. Also, the event reported by McLean and Sheridan⁵ is strongly reminiscent of a short quasi-periodic phase followed by a long decay phase (brought about in our theory by a weak ($\rho_0 \approx 2\rho_e$, say) density difference in the coronal inhomogeneity).

Finally, we note that our treatment is an adiabatic one, dissipative effects being ignored. Conduction and radiative effects are unimportant on the time scale of the observed periodicities. However, viscous dissipation is likely to be important (ref. 22 and J. V. Hollweg, personal communication), and may act as a filter so that only strongly generated disturbances propagate far from the flare site. Additionally, viscous dissipation is least effective in strong field regions²² (say $B > 10 \text{ G}$). Thus, it may be that only shock-generated disturbances in strong field regions are manifest far from the flare site as a pulsation (ref. 4 and J. V. Hollweg, personal communication).

Altogether, then, we have argued that density enhancements in the corona support freely propagating or standing fast waves, which exhibit periodic and quasi-periodic structure. A more detailed investigation²³, incorporating the effects of a non-zero sound speed, is in preparation.

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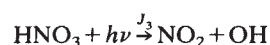
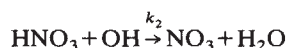
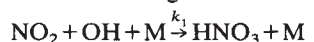
Derivation of OH concentration from satellite infrared measurements of NO₂ and HNO₃

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The hydroxyl radical is a very important radical in the chemistry of the atmosphere. In the stratosphere it is involved not only in the catalytic destruction of ozone but also in the interaction of the nitrogen, chlorine and hydrogen families and in the partitioning of individual species within these families¹. A thorough knowledge of the distribution and behaviour of OH is therefore very useful. However, OH is difficult to measure and only a few published profiles²⁻⁴ exist, confined to 32 °N. The ability to infer global OH fields from direct measurements of NO₂ and HNO₃ would therefore be a major advance in the study of the stratosphere. Geophysical quantities (for example, geostrophic winds, eddy heat and momentum fluxes) have been derived indirectly from satellite data and have proved useful in understanding the behaviour of the stratosphere and mesosphere. The advent of satellite measurements of various trace gases by the stratospheric and mesospheric sounder (SAMS) (CH₄, N₂O, H₂O, NO and CO) and the limb infrared monitor of the stratosphere (LIMS) (O₃, NO₂, HNO₃ and H₂O) now allows us to derive further chemical quantities. Here we present for the first time a cross-section of OH in the middle stratosphere derived from satellite measurements of NO₂ and HNO₃ by the LIMS instrument flown on Nimbus 7. The values obtained agree well with the limited number of previous direct measurements and the variation of derived OH with height and latitude agrees well with model predictions. Further calculations are being made to derive the seasonal behaviour of a radical of central importance in the chemistry of the middle atmosphere. The present calculations nevertheless demonstrate the great power of coordinated satellite measurements.

In the upper stratosphere, NO₂ and HNO₃ are in equilibrium to a good approximation through the reactions



and we can write

$$\frac{[\text{NO}_2]}{[\text{HNO}_3]} = \frac{J_3 + k_2[\text{OH}]}{k_1[\text{OH}][\text{M}]} \quad (1)$$

Thus, with knowledge of the concentrations of NO₂ and HNO₃ and J_3 , k_1 and k_2 , respectively the photolysis rate and rate constants, OH can be calculated.

We have derived OH distributions using all the daytime LIMS NO₂ and HNO₃ data (using the archived retrieval) for 6 days during 1979. The rate constants were taken from WMO (1982)¹, with the photolysis rates calculated using the recommended spectral data. The instantaneous value of J_3 was calculated for every retrieved profile using the appropriate zenith angle and LIMS O₃ and temperature data. Scattering was not included in the calculation of J_3 but this should not introduce serious errors in the region of interest above 30 km (refs 5-7). Individual OH profiles were then computed.

Before presenting the results, we should consider the accuracy expected in our derivation of OH. First, equation (1) will only be a good approximation when the time constant for equilibrium is short. The time constant is of the order of 1 day throughout the stratosphere. Although this might seem unreasonably long, this will only be the case when the ratio HNO₃/NO₂ is perturbed significantly from that implied by equation (1). Our method was checked using a diurnal photochemical model⁸. OH was calculated from the model HNO₃ and NO₂ values using equation (1) and then compared with the model OH (which was calculated from its sources and sinks). For the limited number of model points at which we were able to make the comparison, we found only small differences, ranging at about 28 °N from ~10% at 25 km to 5% at 40 km. We intend to perform a detailed investigation with the diurnal model in due course but these preliminary checks suggest that derivation of OH from equation (1) is entirely appropriate.

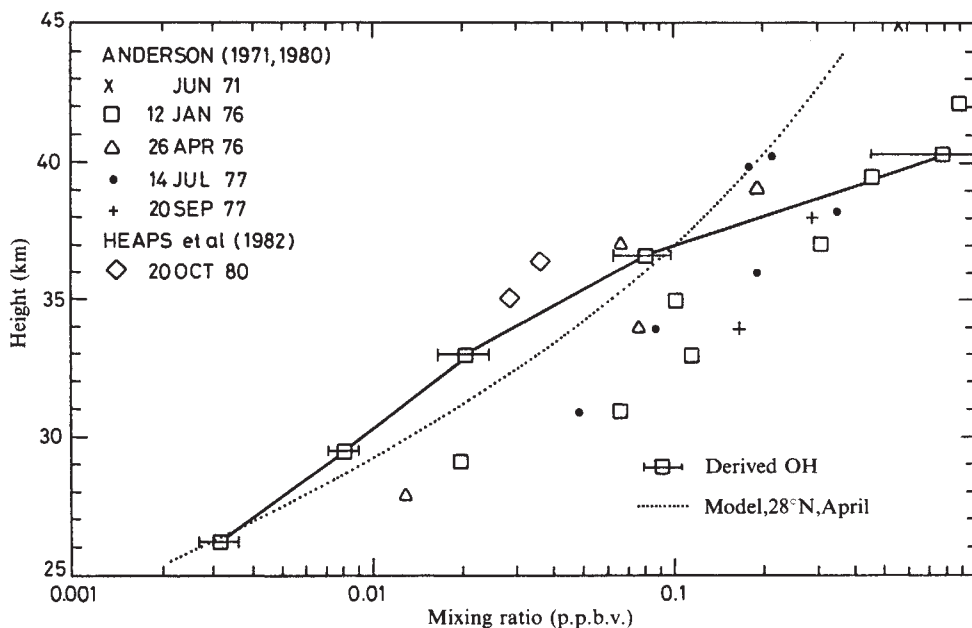
Equation (1) could be integrated over the equilibrium lifetime to derive a mean OH, but with a Sun-synchronous orbit the satellite coverage does not allow this. However, equation (1) was also solved using photolysis rates averaged over the sunlit hours and an average HNO₃/NO₂, found by modifying the instantaneous, observed data in accordance with the diurnal model results. As expected, this produced results less, by about a factor of 2 at 32 °N, than the instantaneous values.

The length of the time constant does introduce an uncertainty into our derivation which should be borne in mind when considering our results. Ideally we would like sufficient satellite data with a fine temporal resolution so as to overcome the problems associated with limited sampling by a Sun-synchronous orbiter. Perhaps the best hope for an independent test of our method lies with concurrent balloon measurements of NO₂, HNO₃ and OH.

Second, we should consider the errors introduced by uncertainties in the data themselves. We follow the error analysis presented by Harries⁹. If we assume an error of ~±25% in both NO₂ and HNO₃, with no errors in the other parameters, we should expect an error in OH of ~±40%. On the other hand, if we assume equal errors in all the parameters and demand a ±50% error in OH, then measurements of NO₂ and HNO₃ are required to ~±20%. ±25% is the best accuracy quoted by the LIMS experimenters^{10,11}, based on estimates of various error sources. However, they point out that, for example, differences between *in situ* and LIMS HNO₃ are less than would be expected based on their analysis, and, furthermore, that the ratio of NO₂/HNO₃ agrees reasonably well with previous measurements. Moreover, factors which contribute to errors in both NO₂ and HNO₃, such as errors in the temperature retrieval, in the field of view of the instrument and in the retrieval algorithm, might be expected to cancel out when the ratio of HNO₃/NO₂ is taken (and note that the k_2 term makes only a small contribution in equation (1)). It is therefore reasonable on this basis to accept a value of ±40% as the uncertainty in OH, although we believe this error can be considerably improved by further study.

Figure 1 shows an average OH profile obtained from 28 profiles around 32 °N derived using the daytime LIMS data for 27 March-1 April 1979. The bars indicate simply the standard deviation of the 28 cases; this presumably arises from errors in

Fig. 1 Derived OH at 32°N for 27 March–1 April 1979 compared with available *in situ* observations and a numerical model calculation. Mixing ratio is expressed in parts per 10^9 by volume (p.p.b.v.).



the measured NO_2 and HNO_3 as well as from atmospheric variability. Systematic errors, arising either from our method or in the data, are not included. The altitude range plotted covers the complete region of overlap of the NO_2 and HNO_3 measurements. Also plotted are the published stratospheric *in situ* measurements²⁻⁴, all made at 32°N during various times of the year. The low zenith angle measurements of Anderson have been modified to midday conditions. The agreement between the derived OH and the measurements is very encouraging, especially in the region above 35 km where we believe our method is most accurate. In the lower stratosphere the LIMS-derived OH falls below the *in situ* measurements. The significance of this is unclear due to the small number of measurements, with the attendant problem of how representative these are.

Figure 1 also gives a model profile calculated for 28°N using a two-dimensional model¹² with kinetic data from WMO (1982)¹. The profile represents a day average and would be expected to underestimate the midday values by a factor of ~ 2 . When this is considered we regard the agreement, particularly in the upper stratosphere, as good.

Of particular interest is the difference between the derived OH and model values in the lower stratosphere, for it is this region, above ~ 25 km, that many models overestimate the HNO_3 profile¹. These two factors possibly point to an inadequacy in our knowledge of the chemistry of this region. Scattering has not been included in the calculation but this would increase J_3 , and hence OH, by only $\sim 10\%$ at 25 km and by much less at 35 km. Clearly, more measurements of OH by different techniques are needed to answer the questions about the region between 25 and 25 km. As a complementary study we are attempting to derive OH from measurements of its sources (O_3 , and hence $\text{O}(^1\text{D})$, and H_2O) and sinks (which will be modelled).

To understand the chemistry of the stratosphere, we ideally require measurements with a wide spatial and temporal coverage. A great advantage of our method is that nearly-global fields of OH can be derived. Figure 2 is a zonal mean cross-section of daytime OH for 27 March–1 April, compared with the two-dimensional model field of OH for April. In producing the plot, individual derived OH profiles have been averaged in 4° latitude bins. No smoothing has been applied. The continuity of the results as a function of latitude is remarkable and, we feel, lends considerable support to our method as it indicates little random variability in the derived OH field.

The derived OH peaks in the upper stratosphere. The concentrations decrease with latitude, especially in the low stratosphere.

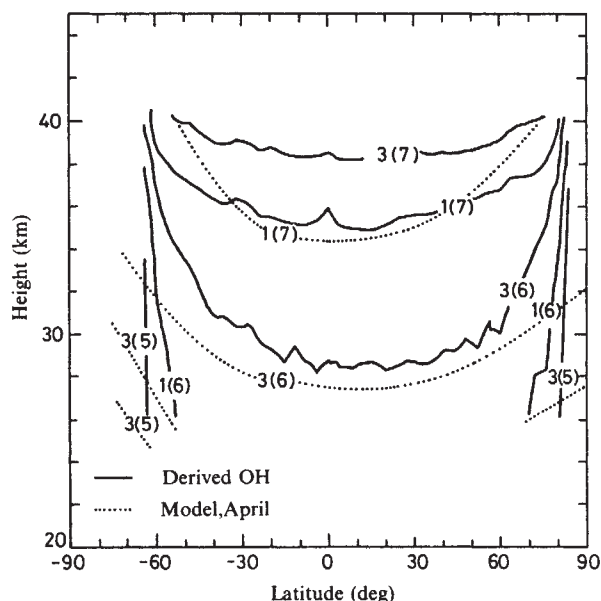


Fig. 2 Cross-section of derived OH concentration (mol cm^{-3}) for 27 March–1 April compared with a model cross-section for April ($3(6) = 3 \times 10^6$).

phere. The comparison with the modelled fields is generally good, with the slopes of the contours agreeing well.

It is possible that the satellite coverage accentuates the latitudinal gradients in high latitudes because the measurements are not all made at the same local time. Between approximately 30°S and 60°N the LIMS measurements are made at approximately 1300 h local time and the derived OH should be directly comparable. Approaching 60°S , the measurements are taken at about 1500 h, when diurnal model calculations⁸ indicate that OH would be reduced by a factor of about 2 from its noon value.

Despite the reservations expressed above concerning the lower stratosphere, we nevertheless believe that the simple technique outlined here is a new and potentially extremely useful method for providing global information, at present not available from other methods, about a most important atmospheric constituent. By virtue of the spatial and temporal coverage it is capable of revealing very significant information about latitudinal and seasonal variations which is unlikely to be provided by conventional techniques for many years. Following the encouraging demonstration of this new technique, derivation of

OH for the whole of the LIMS dataset is underway. Finally, we stress that our calculations demonstrate the great power of coordinated satellite measurements of complementary species as made by instruments like LIMS and SAMS. In this context the experiments to be flown on the Upper Atmosphere Research Satellite offer exciting research opportunities.

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Fluid flow along a channel with an asymmetric oscillating constriction

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We present here experimental results and theory on the flow of an incompressible fluid (water) in a two-dimensional closed channel with an oscillating asymmetric constriction (Fig. 1). The motion of the constriction models the large amplitude self-excited oscillations that arise when fluid flows through a collapsible tube such as a vein^{1,2}. Downstream of the oscillating constriction, a train of waves appears in the core flow and a double row of eddies forms along the channel walls (Fig. 2). This phenomenon, which occurs at all but the lowest frequencies, has not previously been observed. Our theory explains the occurrence of the wavetrain and accurately predicts several of its properties.

The 100×10 mm channel was adapted from one used in an earlier study on impulsively-started flow past a fixed constriction³. In the present study the constriction is a thick rubber membrane which is pushed from behind by a sinusoidally oscillating 100×100 mm piston; the retracted position of the membrane is flush with the channel wall. The flow upstream of the constriction is fully developed and the flow rate is kept constant by a control valve. The fluid motion in the channel is made visible by light reflecting pearl essence particles known as 'Mearlmaid AA'. Photographs and motion pictures of the flow are taken at right angles to a plane of light which illuminates the channel centreplane; the photographs are synchronized with the driving piston.

The governing parameters for the flow are: the Reynolds number $Re = a\bar{u}_0/\nu$, where a is the undisturbed channel width, $\bar{u}_0$ is the mean velocity, and ν is the kinematic viscosity of the fluid; the Strouhal number $St = a/T\bar{u}_0$, where T is the period of oscillation; and the amplitude parameter ε ($0 \leq \varepsilon \leq 1$) where εa is the maximum constriction height. The values of the parameters in our experiments were: $300 \leq Re \leq 700$, $0.003 \leq St \leq 0.07$, and $\varepsilon = 0.28, 0.38, 0.41, 0.57$. We also observed the steady case ($St = 0$).

When the constriction is fixed a single separated eddy is observed along its downstream sloping wall and, for sufficiently large Reynolds numbers ($Re > 500$) and severe constrictions, an additional small eddy is present along its upstream sloping wall. When the constriction is oscillating slowly, with $St < 0.008$, a flow field similar to the steady flow field is observed. However, at higher frequencies of oscillation, $St \geq 0.008$, waves develop on the core flow downstream of the constriction and eddies appear along the channel walls.

Figure 2 gives photographs of a typical cycle for $St > 0.008$ showing the formation of the eddies along the channel walls, their propagation downstream and the subsequent return to undisturbed flow. The times t are non-dimensionalized by the period T so that there are two phases in which the piston is accelerating, $0 \leq t < 0.25$ and $0.50 \leq t < 0.75$ and two deceleration phases, $0.25 \leq t < 0.50$ and $0.75 \leq t < 1.00$, in the cycle. During the first acceleration phase, as the membrane is pushed into the channel from its flush position, a single eddy forms and grows along the downstream wall of the constriction. While the membrane is still advancing into the channel but is decelerating (Fig. 2a–c) a second eddy appears on the wall opposite the constriction. There is evidence of the separation that leads to the second eddy at $t = 0.34$ (Fig. 2a) and by $t = 0.41$ (Fig. 2b) the second eddy, which is downstream of the first eddy, is clearly visible. As the second eddy grows and moves downstream, a third eddy forms on the wall with the constriction (Fig. 2c, d) and subsequently a fourth eddy forms on the plane wall (Fig. 2d, e). All the eddies move downstream, at a speed which is much less than both $\bar{u}_0$ and the speed at which the front of the system of waves and eddies propagates into the undisturbed flow. The wavelength of the waves is observed to increase as either $\bar{u}_0$ or T is increased (that is, St is decreased). After the fourth eddy appears, two additional eddies are visible upstream of the second (Fig. 2e) and third eddy (Fig. 2f), respectively, as if these eddies had split into two; the eddies in each pair have the same direction of rotation. In Fig. 2g–i, the membrane is decelerating to its flush position. During this phase all the eddies are swept downstream and begin to lose their structure. The flow then returns to an undisturbed state.

The observations on steady flow are in agreement with the theoretical predictions of Smith and Duck⁴. The flow field about the slowly oscillating constriction, with $St \leq 0.008$, is similar to the flow field past the fixed constriction and may be regarded as quasi-steady. Quasi-steady flow was also observed in the earlier study on impulsively-started flow past a fixed constriction³, for times corresponding to $St \leq 0.013$. On the other hand, in a study on oscillatory flow in a furrowed channel, at smaller values of Re , it has been shown both numerically⁵ and experimentally⁶ that the flow is quasi-steady only in the acceleration phase, not the deceleration phase, for $0.001 < St < 0.02$.

For $St \geq 0.008$, the flow through the channel is not quasi-steady. Physically, the time-dependent displacement of the flow by the piston and the first eddy generates a cross-channel pressure gradient that causes curvature in the main stream which leads to the generation of the second eddy. With the formation of the second eddy, a cross-channel pressure gradient in the opposite sense is created, and the process repeats itself with the formation of the third and fourth eddies. This process is essentially inviscid, although the details of the boundary layers, separation and eddy formation clearly involve viscous effects, which can be incorporated in an asymptotic theory for $Re \gg 1$, at least if $\varepsilon \ll 1$ (refs 4, 7, 8). We now present a theory for the waves on the coreflow in which viscosity is neglected; this is equivalent to the assumption that the eddies are passive structures whose existence does not affect the dynamics of the core until their amplitude is so large as to block the flow (Fig. 2h, i). The 'eddy-splitting' is a phenomenon that cannot be explained at present, as it is not predicted by the theory. Cine films of the flow show that the core flow flutters just before the inception of the additional eddies, which suggests that they may be the consequence of a shear layer instability.

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Fig. 1 Sketch, at the channel mid-plane, showing the section of the channel with the constriction. The depth of the channel in the direction perpendicular to the sketch is 100 mm. The dimensionless coordinates x, y are shown in the sketch.

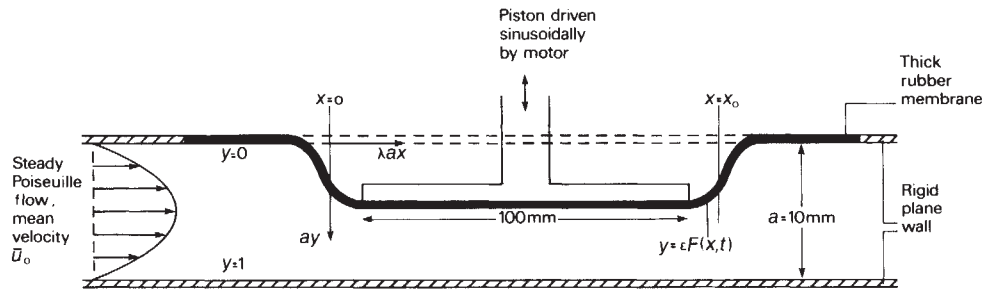
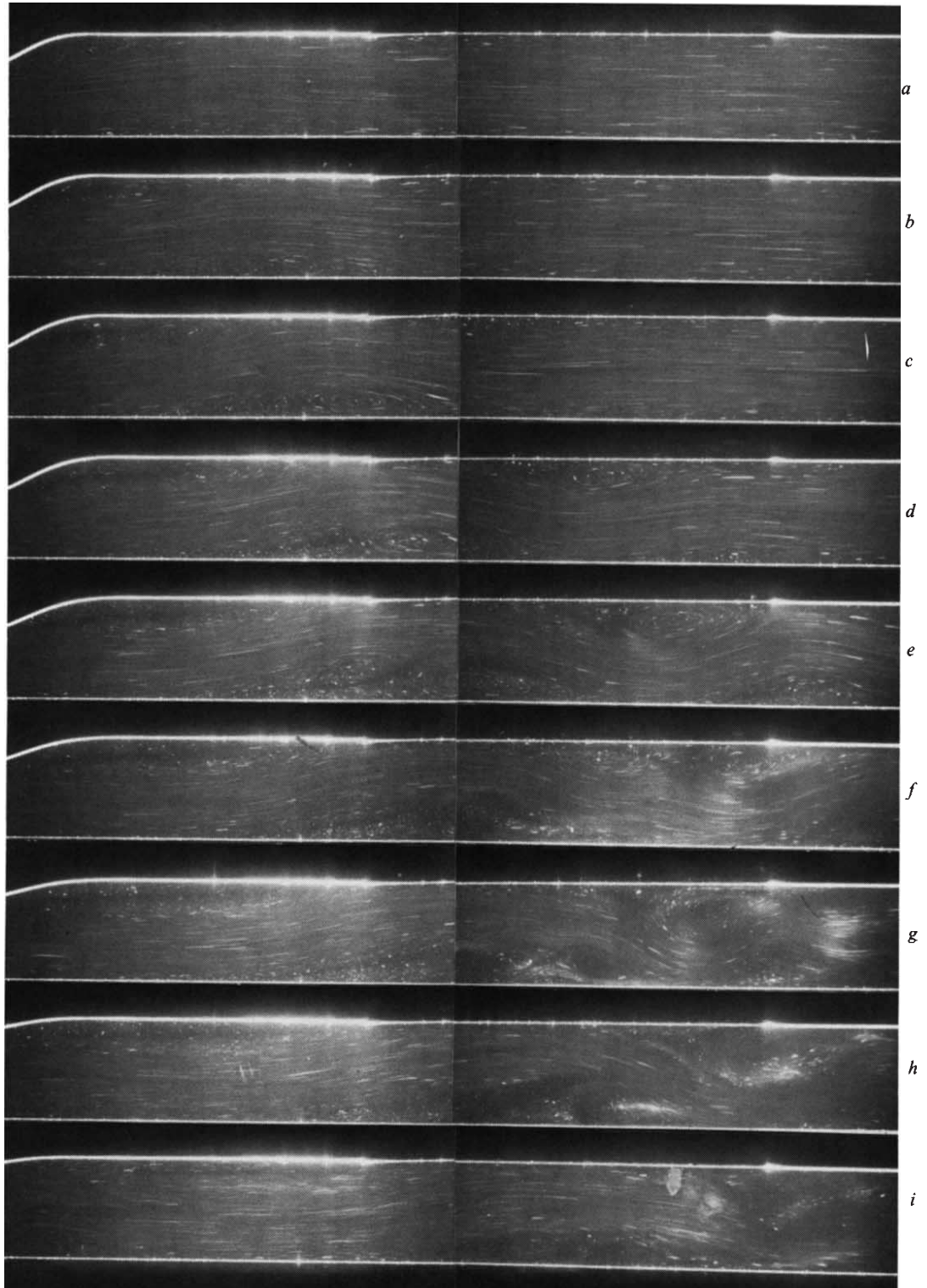


Fig. 2 Photographs showing the development of the flow field downstream of the oscillating constriction. The mean flow is from left to right and the governing parameters are: $Re \approx 610$, $St = 0.038$ and $\epsilon a = 3.8$ mm. The times of the photographs are: *a*, $t = 0.338$; *b*, $t = 0.409$; *c*, $t = 0.480$; *d*, $t = 0.551$; *e*, $t = 0.622$; *f*, $t = 0.693$; *g*, $t = 0.764$; *h*, $t = 0.835$; *i*, $t = 0.906$. The second eddy is first visible in *b*, the third in *c* and the fourth in *d*. An additional eddy appears upstream of the second eddy in *e* and upstream of the third eddy in *f* (eddy-splitting).



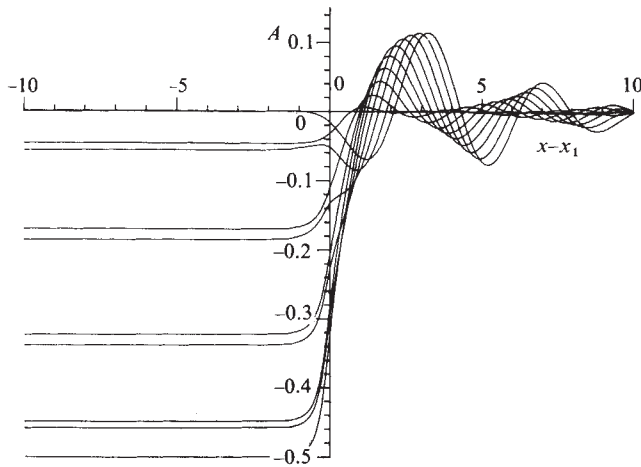


Fig. 3 Predictions of A as a function of x for $t = 0.099$ – 0.990 in steps of 0.099 ; $\varepsilon = 0.38$, $St = 0.057$.

Let a longitudinal length-scale be λa , where $\lambda \gg 1$ but $\lambda \ll Re$ and introduce dimensionless variables (t, x, y, u, v, p) as follows: time Tt ; coordinates $a(\lambda x, y)$, with the channel boundaries at $y = 1$ and at $y = \varepsilon F(x, t)$ where $F = 0$ for $x \leq 0$ and $x \geq x_0$ (say), and where $\max[F(x, t)] = 1$ for $0 < x < x_0$ (Fig. 1); velocity components $\bar{u}_0(u, v/\lambda)$; pressure $\rho \bar{u}_0^2 p$, where $\rho = \text{fluid density}$. We require that there is steady Poiseuille flow as $x \rightarrow -\infty$, that is

$$u \sim U(y) \equiv 6y(1-y) \quad (1)$$

and $p \sim 12\lambda x/Re$. When scaled in this way, the two-dimensional Navier–Stokes equations are as follows, where a suffix represents partial differentiation:

$$\text{conservation of mass: } u_x + v_y = 0 \quad (2)$$

$$\text{x-momentum: } \lambda St u_t + uu_x + vv_y = -p_x + 0(\lambda Re^{-1}) \quad (3)$$

$$\begin{aligned} \text{y-momentum: } \lambda^{-1} St v_t + \lambda^{-2}(uv_x + vv_y) \\ = -p_y + 0(\lambda^{-1} Re^{-1}) \end{aligned} \quad (4)$$

The error terms in equations (3) and (4) are the viscous terms, negligible if $Re \gg \lambda$. The boundary conditions, purely kinematic, are $v = 0$ on $y = 1$ and

$$v = \varepsilon(uF_x + \lambda St F_t) \quad \text{on } y = \varepsilon F(x, t) \quad (5)$$

We now assume $\varepsilon \ll 1$ and expand the variables in powers of ε , so that the leading term in u is the undisturbed flow $U(y)$. We also take the Strouhal number to be small, $\ll \lambda^{-1}$ (formally, in fact, we shall take $St = 0(\lambda^{-3})$). This means that the first correction to the undisturbed flow is quasi-steady, and is simply derived from the leading, $0(\varepsilon)$, terms of equations (2), (3) and (5). Thus we have

$$\begin{aligned} u &= U(y) + \varepsilon A(x, t)U'(y) + \varepsilon^2 u_2(x, y, t) + \dots \\ v &= -\varepsilon A_x(x, t)U(y) + \varepsilon^2 v_2(x, y, t) + \dots \\ p &= \varepsilon^2 p_1(x, y, t) + \dots \end{aligned} \quad (6)$$

where $A(x, t)$ is the as yet unknown displacement function. The first term in equation (4) gives

$$p_1 = P(x, t) + \lambda^{-2} \varepsilon^{-1} A_{xx} \int_0^y U^2(y') dy' \quad (7)$$

showing how the transverse pressure gradient is proportional to the curvature A_{xx} of the displaced streamlines; the function $P(x, t)$ is unknown (see ref. 8). Formally, we take $\lambda = 0(\varepsilon^{-1/2})$ so that the two terms of equation (7) are of comparable magni-

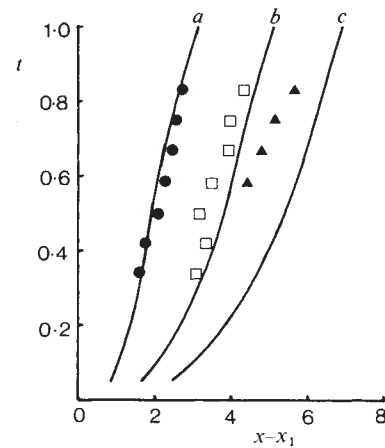


Fig. 4 Predicted and measured positions of the stationary points of A , plotted against t , for one experiment; $\varepsilon = 0.38$, $St = 0.057$. Curves: predictions; points: experiment; a, ● first maximum; b, □ first minimum; c, ▲ second maximum. The first photograph was taken at $t = 0.34$, by which time both the first maximum and first minimum had appeared.

tude. Finally, we take the $0(\varepsilon^2)$ term in equation (3), which gives

$$\begin{aligned} \lambda St \varepsilon^{-1} A_t U' + A A_x U'^2 + U u_{2x} - A A_x U U'' + U' v_2 \\ = -P_x - \lambda^{-2} \varepsilon^{-1} A_{xxx} \int_0^y U^2(y') dy' \end{aligned} \quad (8)$$

The y -dependence of u_{2x} and v_2 is unknown, but the coefficient of u_{2x} is zero at $y = 0$ and $y = 1$ and v_2 can be evaluated at each wall from the boundary conditions. (Although expansion (6) is not uniformly valid as $y \rightarrow 0$ or 1 , because U is zero there and U' is not, it can be shown that the critical layers required there do not affect v_2 at this order^{4,7}.) Evaluating equation (8) at $y = 1$ and at $y = \varepsilon F$ (using equation (5)), and eliminating P , gives

$$A_{xxx} - 2\omega A_t = \omega F_t + \varepsilon_1 (FF_x + AF_x + FA_x) \quad (9)$$

where

$$\omega = 5\lambda^3 St, \quad \varepsilon_1 = 30\lambda^2 \varepsilon$$

and both are $0(1)$ by hypothesis. This equation has earlier been derived without the ε_1 term⁷; the corresponding steady equation has been derived with the ε_1 term⁴.

Equation (9) is a nonlinear partial differential equation for the displacement function $A(x, t)$. We note that the condition of no disturbance ($A \rightarrow 0$) as $x \rightarrow -\infty$ means that $A \equiv 0$ for $x < 0$. We also note that in the downstream region where $F \equiv 0$ ($x > x_0$), the right-hand side of equation (9) is zero, and the equation reduces to the linearized Korteweg-de-Vries equation. This permits the propagation of waves with (dimensional) phase velocity $\frac{1}{10} \bar{u}_0 a^2 k^2$, where k is the wavenumber, and with group velocity three times as large. This is qualitatively consistent with the observation that the front of the wave train propagates much more rapidly than the crests.

We have performed numerical integrations of equation (9) for the case in which $F(x, t)$ is separable, $= f(x)g(t)$, where

$$f(x) = \frac{1}{2} \{1 - \tanh[\alpha(2\omega)^{1/3}(x - x_1)]\}, \quad g(t) = \frac{1}{2} (1 - \cos 2\pi t)$$

This shape approximately represents the right-hand end of our flat constriction, with $x = x_1$ at the midpoint of the downstream slope. We ignore the left-hand end of the constriction because any disturbances introduced there are not observed at the right-hand end. It is convenient also to choose $\lambda = (10 St)^{-1/3}$ so that $2\omega = 1$ and $\varepsilon_1 = 30\varepsilon/(10 St)^{2/3}$. Parameter values have been chosen to match one of our experiments: $St = 0.057$, $\varepsilon = 0.38$ (so $\varepsilon_1 = 16.6$) and $\alpha = 5$, representing a realistically steep downstream slope ($f(x) = 0.01$ where, dimensionally, $x - x_1 = 6.4$ mm). Figure 3 shows how the computed displacement function $A(x, t)$, changes with time during the first cycle, $0 < t < 1$.

We see that a wave is generated, grows, and propagates downstream, as observed except that eddy-splitting is not predicted. To make quantitative comparison with experiment, we have plotted the locations of the first few wave crests as functions of time. This plot, for the same parameter values as Fig. 3, is given in Fig. 4, which shows that the positions of the first maximum and minimum of A are accurately predicted by the theory, although the value of x at the second maximum is overestimated. These results suggest that our theory contains the essential features of the observed flow.

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Effect of acceleration on sea ice growth

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Field studies¹ and laboratory experiments^{2,3} have shown that sea currents align the crystallographic c axes in sea ice, illustrating the profound effect of fluid motion on the freezing of brine. The role of buoyancy forces in the melt is further investigated here by solidifying NaCl solution in a centrifuge. When the ice is grown under negative accelerations (choosing the solidification direction as positive) the salt concentration in the ice decreases monotonically with depth, whilst with positive acceleration it has the characteristic C-shaped profile found in natural sea ice. In addition the concentration decreases as the acceleration increases at any given depth. This behaviour has been found to be due to variations in the initial trapping of brine rather than to subsequent buoyancy-driven drainage. Accelerations up to 250 ms^{-2} ($25.5g$) reduce the scale of features within the 'solid' while maintaining the essential characteristics of a sea ice cover.

There are several reasons for examining the changes in the physical properties of a material with a change in acceleration. In soil mechanics, for instance, some phenomena can be correctly scaled by placing models in a centrifuge, thereby increasing self-weight effects in a test⁴. A further example is found in materials processing where it has been suggested that imperfections due to buoyancy-driven convection might be avoided if the material was grown in space. This has prompted theoretical^{5,6,7} and experimental^{8,9} investigations of solidification at reduced accelerations, and laboratory studies have been extended to accelerations greater than Earth's gravity^{9,10}. In addition the centrifugal washing of brine from frozen seawater has been explored as a method of desalination to produce drinking water¹¹.

The freezing of seawater is similar in many ways to the solidification of metal and semiconductor alloys. The impurity is rejected at the ice/water interface and diffuses into the liquid at a slower rate than the diffusion of heat to the interface. This causes the liquid immediately ahead of the interface to be below its equilibrium freezing point; that is, it is said to be constitutionally supercooled. A protrusion on the interface therefore

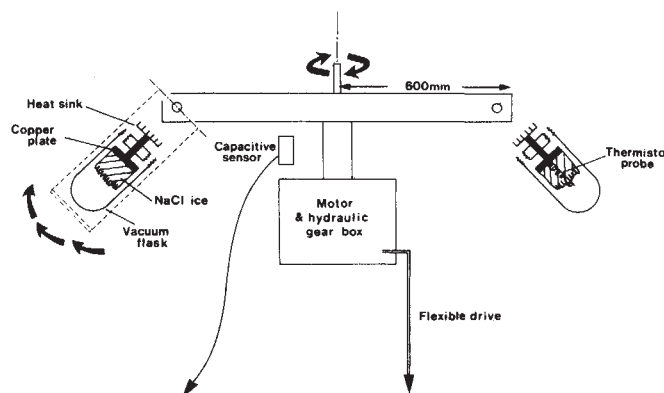


Fig. 1 Schematic diagram of the centrifuge constructed in a cold room. Brine of a salinity typical of Arctic waters (27‰) was frozen in vacuum flasks of 76 mm diameter. The flasks are ideal for modelling sea ice growth since they produce unidirectional solidification and low temperature gradients in the liquid. Heat sinks and a thick copper plate at the upper ice surface ensured rapid heat transfer to the air at -25°C . Flasks rose up under the centrifugal acceleration so that the acceleration and direction of solidification were coincident. The magnitude of the acceleration was deduced from the frequency of passage of the arm over a capacitive sensor. A string of 5 thermistors at 20-mm intervals recorded the increase in thickness with time and the cooling rate in the ice. The ice was removed when it was approximately 65 mm thick.

finds itself in a region of supercooled water and the growing interface becomes an array of cells or dendrites. Brine is trapped in the intervening grooves and the resulting 'solid' consists of a laminate of high purity ice separated by pockets of brine.

In nature, sea ice freezes downwards with the heat flowing unidirectionally upwards to the heat sink at the ice surface. Thus the direction of growth and the gravitational acceleration are coincident. In these conditions there is a layer of cold, saline brine at the ice/water interface which is denser than the underlying liquid and buoyancy-driven convection takes place. The interface develops into a regular array of two-dimensional cells, called platelets, each of which is elongated perpendicular to the c axis. These axes rapidly swing round from fairly random orientations at the ice surface to within a few degrees of the horizontal plane at greater depths. Grain size and platelet spacing increase with depth while the amount of salt trapped in the 'solid' decreases. These observations can be related to the decrease in solidification velocity due to the insulating effect of the ice.

The study of the formation of sea ice has therefore meant that there have been numerous investigations of the case where the growth velocity is parallel to the acceleration. However, solute segregation¹² and interface morphology^{13,14} have also been observed for the upward growth of impure ice. In this case, the cold, saline brine of the interface causes the density profile in the fluid to be stable and no buoyancy-driven convection occurs.

The present investigation was carried out on the apparatus shown in Fig. 1. In all tests the rate of heat extraction was sufficiently great that the solidification velocity was independent of the velocity of the flasks through the air. In addition variations in cooling rate between experiments were less than 10%. Since these are major parameters in determining the material properties¹⁵ we are confident that any residual effect is due to acceleration. The magnitude of this acceleration varied by about 10% through the thickness of the sample. Errors introduced by the Earth's acceleration and the Coriolis effect on the draining brine are negligible.

For all experiments the macroscopic interface was flat, indicating that radial heat flow had been sufficiently reduced by the copper plate. However, there were significant differences between the interface morphologies, particularly in the transition from experiments at positive accelerations to those at

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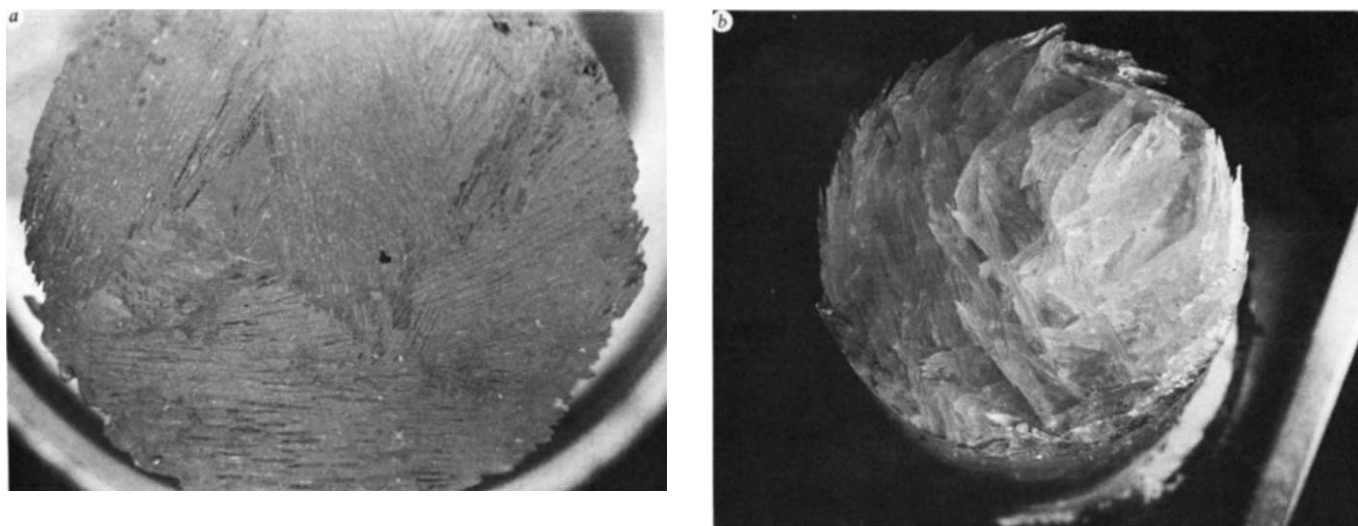


Fig. 2 *a*, The ice/water interface commonly observed under sea ice, that is at +1g. Note the elongated platelet structure. The *c* axis is perpendicular to the long axis of these platelets. Distances from centre to centre of neighbouring platelets, called the platelet spacing, are approximately 0.5 mm. *b*, Open dendritic interface of NaCl ice grown at -1g. *c* axes are perpendicular to the planes of the dendrites. Sample diameter, 75 mm.



Fig. 3 Vertical thin sections photographed in transmitted light between crossed polaroids. Samples extend from the copper plate to the bottom of the NaCl ice for *a*, +1g; *b*, +10g; *c*, +26g; *d*, -1g; *e*, -12g; *f*, -31g. The *c* axis of grains is perpendicular to their long axis. Sample widths, 75 mm; scale bar, 10 mm.

negative accelerations. In the positive regime, a regular array of two-dimensional cells developed which is characteristic of natural sea ice (Fig. 2a). However, the depth of the intercellular grooves decreased as the acceleration increased. In the negative regime the structure becomes fully dendritic (Fig. 2b), with deep grooves extending well behind the growth front. The presence of these grooves forms large voids within the ice. The vertical thin sections (see Fig. 3a-f) show that columnar growth extends from the copper plate to the ice/water interface in all cases. In addition, grains which were nucleated at the copper plate with c axis close to the vertical are generally eliminated in favour of those with c axis horizontal. However, the rate at which this reorientation is achieved increases as the acceleration is increased from the negative through the positive regime.

Horizontally-averaged salinity measurements were taken through the ice thickness and are shown as a function of dimensionless depth for a number of accelerations in Fig. 4a. The characteristic C-shaped profile of sea ice¹⁶ can be seen in all the positive acceleration runs. At negative accelerations the concentrations are significantly higher and decrease monotonically with depth. It is interesting to note that close to the copper plate in growth at $-31g$, (denoting Earth's gravity by g) the salinity appears to reach saturation.

The general trend is for the amount of solute present in the 'solid' to decrease with increasing convection, a result also deduced by Kvačić *et al.*¹² from more limited data at $+1g$ and $-1g$ and by Johnson and Parr⁹ for tin-lead alloys. To explain this we examine the variations in initial trapping of brine in the structure of the impure ice. The trapping of solute at a planar interface when there is a diffusive boundary layer in the fluid of thickness, δ , has been derived by Burton *et al.*¹⁷ as

$$k_{\text{eff}} = \frac{k_0}{k_0 + (1 - k_0) \exp(V\delta/D)} \quad (1)$$

where V is the growth velocity and D is the diffusion coefficient of solute in the liquid.

$$k_0 = \frac{C_s}{C_i} = \text{equilibrium segregation coefficient}$$

$$k_{\text{eff}} = \frac{C_s}{C_\infty} = \text{effective segregation coefficient}$$

where C_s is the concentration of solute in the solid, C_i is the concentration at the interface and C_∞ is the concentration in the bulk of the liquid. Although this equation is not strictly applicable for cellular growth, it has been used with success to describe solute segregation in sea ice growth^{18,19}. We therefore use equation (1) with the empirically determined value^{18,19} of $k_0 = 0.26$ to find the diffusion boundary layer as a function of acceleration (Fig. 4b). The values calculated for δ are within the range measured by Terwilliger and Dizio²⁰ for the downward freezing of NaCl solutions at similar growth rates to the present experiments.

For the positive accelerations, the rejection of impurities mixes the fluid provided the solute Rayleigh number defined as

$$Ra = \frac{\beta a \Delta C h^3}{D\nu} \quad (2)$$

exceeds the critical value of the order of 10^3 . In this equation, a is the acceleration, β is the coefficient of haline expansion, ν is the fluid viscosity and ΔC is the concentration difference over a layer of thickness h . Following Wimbush and Munk²¹ we propose that the diffusive boundary layer extends to the depth at which Ra attains its critical value. Thus

$$\delta \sim \left\{ \frac{10^3 D \nu}{\beta \Delta C a} \right\}^{1/3} \quad (3)$$

As shown in Fig. 4b the data follow the curve $\delta = 420a^{-1/3}$. The constant of proportionality suggests that $\Delta C \sim 10\%$ which is of the same order of magnitude as previous estimates of the salinity excess of rejected brine^{22,23}.

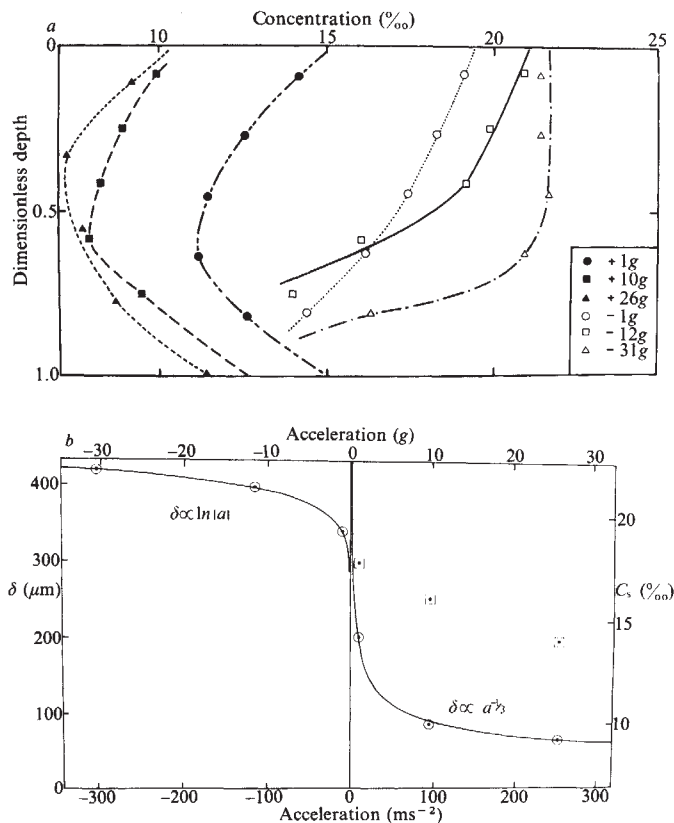


Fig. 4 *a*, Concentration of solute in NaCl ice samples as a function of dimensionless distance from the copper plate. Note the decrease in concentration with increase in the intensity of convection in the melt. *b*, S-shaped curves of concentration of solute in NaCl ice samples and the thickness of a diffusive boundary layer as a function of applied acceleration ($\circ$). Only data closest to the copper plate are shown, since the effects of brine drainage after samples are removed from the flasks is minimum at these points. Platelet spacings ($\square$) are also shown for the positive accelerations on the same scale as boundary layer thickness. Solid lines are best fits of deduced relationships for each of the positive and negative regimes.

We have already seen that, in the stable fluid regime of the negative accelerations, the solute enclosed in the solid appears to have reached a saturation level close to the copper plate (see Fig. 4a). This is expected since it must always be true that

$$C_s \leq C_i$$

and thus the rate of change of C_s with acceleration must tend to a limiting value. A reasonable description is thus

$$\frac{dC_s}{da} \propto \frac{1}{a} \quad (4)$$

If there is diffusion only in the liquid, if C_∞ is fixed and if the solute concentration is the result of trapping of brine by the structure of the interface, then

$$\delta \propto C_i \propto C_s$$

giving

$$\delta \propto \ln |a| \quad (5)$$

Figure 4b shows that the experimental data closely follow a relationship of this form.

The platelet spacing, λ , is also shown in Fig. 4b for positive accelerations, and is seen to decrease as the intensity of natural convection increases. This is in agreement with previous observations of refining of structure in forced flow² and with the large values for λ for alloys grown in space⁹. A simple model of diffusion in the interdendritic pool of liquid²⁴ can be used to

explain these results. The model predicts

$$\lambda = \left\{ \frac{2D\Delta T}{G_m V} \right\}^{1/2} \quad (6)$$

where G_m is the temperature gradient in the mushy, solid/liquid zone at the interface and ΔT is the constitutional supercooling midway between cell tips. An increase in acceleration increases the mixing in the fluid, thereby decreasing the temperature gradient in the liquid. Therefore if we assume that far from the

interface the temperature in the solid and liquid are fixed, then the temperature gradient in the mushy zone must increase with increasing acceleration. G_m , as deduced from equation (6) with experimental values of λ , increases in an approximately linear manner with acceleration. In the negative regime, the spacing of the dendritic plates is very much more irregular.

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Structure and stratigraphy of Mazagan (El Jadida) Escarpment (West Morocco): first results of Cyamaz diving campaign

The Cyamaz Group*

The Mazagan Escarpment is located about 200 km south-west of Casablanca in the central Atlantic (Fig. 1). It constitutes an almost 3,000 m high cliff between the Mazagan Plateau and the Seine Abyssal Plain. Because of its steepness, the Mazagan Escarpment provides an exceptional opportunity to study the Mesozoic to Palaeogene stratigraphy, palaeoenvironment and subsidence history of a nearly sediment-free external carbonate platform, exposing the late Jurassic and Cretaceous continental margin sediments of the Atlantic Ocean. Reconstructions of the central Atlantic at the end of the Liassic¹ show the location of the Mazagan Escarpment between the African continent and its thinned, stretched margin, where evaporitic series have been deposited^{2–4}. For several years, many cruises have been conducted in the Mazagan area. Some of the data obtained have been published^{2,5–7}. Before IPOD Leg 79, two DSDP sites (370, 416) have been drilled in the deep Moroccan Basin^{8,9}. Results of Leg 79³ demonstrate the variety of environments during the Jurassic, but also a certain episodicity in the defacing of the scarp up to Neogene times. The Cyamaz diving cruise has been prepared by a bathymetric survey using the multibeam echosounder 'Seabeam' (Fig. 1)¹⁰.

During the Cyamaz cruise, eighteen dives (Fig. 1) enabled us to obtain 130 rock samples and more than 6,000 pictures. The target of the dives was mainly to observe and sample the steepest part of the escarpment where the seismics suggest that the oldest series are exposed. Continuous Seabeam mapping, seismic profiling and direct observation from the submersible allow us to

distinguish three morphological and structural units: (1) The Mazagan Plateau, at a depth of between 500 and 1,300 m of water, shows a very gentle north-west trending slope. In the north-west part, this gentle slope continues until 2,000 m depth. The whole plateau is controlled by NE–SW accidents which correspond to faults visible on the seismic profiles. At depth, a highly reflective horizon probably corresponds to the top of the carbonate platform. Above, there follows a sequence of layered horizons with several seismic units cut by unconformities and erosional surfaces (Fig. 2). They might correspond to the late Cretaceous and Tertiary sequence. (2) The Mazagan Escarpment, made up by a very steep slope between 1,300 to 3,200 m depth in the central and southern part. Along the steepest slopes between 1,800 to 1,400 m depth it is possible to make detailed observations of the lithostratigraphy and structure. In the south the escarpment shows a reentrant feature controlled by NNE–SSW directions and cut by El Jadida Canyon and tributaries. In the north, the escarpment extends down to a depth of 3,500 m. (3) The Mazagan 'Apron', at the foot of the cliff, is very large, grading into Seine Abyssal Plain. The gentle slope is dissected by a series of west–east valleys among which the most important are the El Jadida Canyon in the south and the Azemmour Canyon in the north.

The sedimentary sequence observed along Mazagan Escarpment is summarized in Fig. 3. We noted a fundamental difference between the succession constituting a continuous series along the cliffs and the set of wall exposures 'draping' the slope and resting unconformably on the older series.

Late Jurassic–Neocomian platform limestones have been observed over a lateral distance of about 40 km from 3,000 to 1,400 m depth. They are very massive rocks (Fig. 4a), generally Mn-coated and fractured (open fractures, small normal faults). It is difficult to estimate the vertical displacement, so an evaluation of a total thickness of about 800 m is only an order of magnitude estimate. Such a thickness is compatible with a carbonate platform subsiding at 50 m per Myr during 15 Myr. Sampling along the profiles allowed us to distinguish seven facies types of shallow to deeper water environments: (1) The major rock is a massive pelsparite. The rock shows irregularly distributed pelmicritic layers, which indicate occasional (stromatolitic) algal binding of the sediment. Calcium carbonate supersaturation gave rise to the development of ooids and an early cementation of the normal peloidal sediment. Calcified hexactinellid and lithistid siliceous sponges grew upon the peloidal sediment, but could not form true sponge reefs, as known from European epicontinental seas. (2) Pelsparites with shallow-water benthic foraminifers are less frequent and

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Fig. 1 Seabeam map made after the Seazagan cruise (N.O. J. Charcot, June 1982).

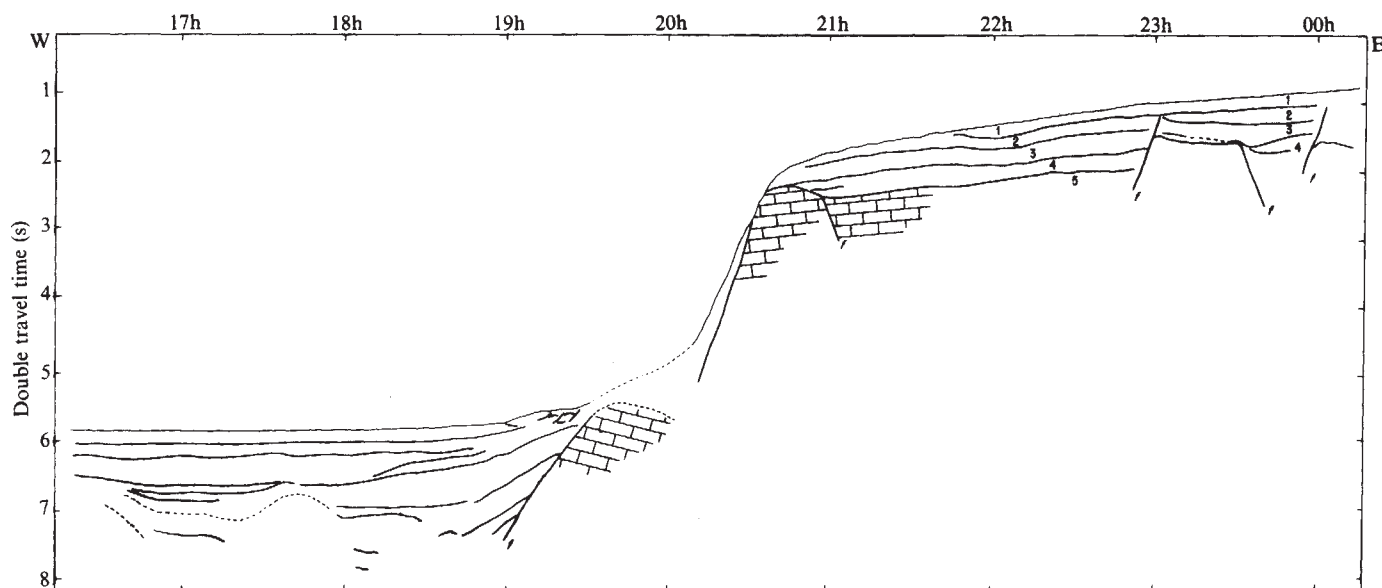
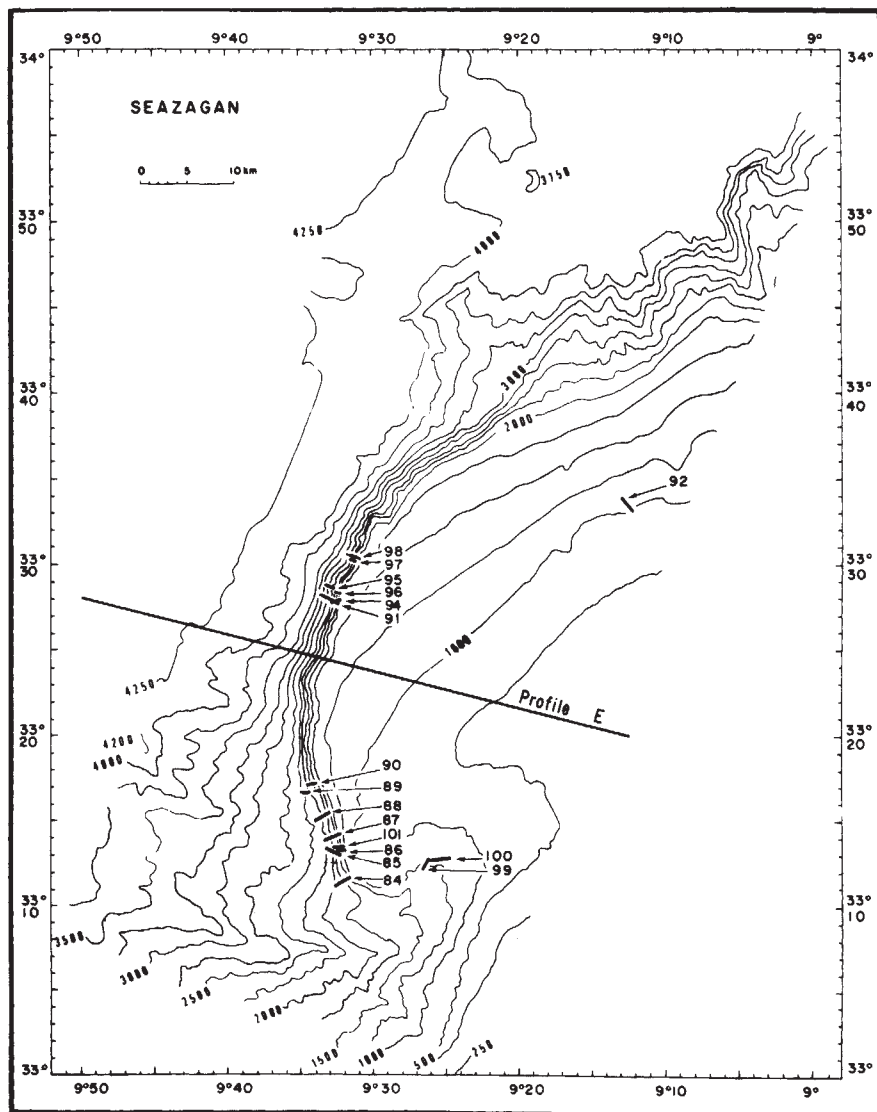


Fig. 2 Single-channel seismic profile (Seazagan profile E, location on Fig. 1). 1, Neogene; 2, Palaeogene; 3, Middle to Upper Cretaceous; 4, Lower Cretaceous; 5, Upper Jurassic-Lower Neocomian Platform; f, fault. The profile was taken on 7/8 June 1982. Vertical exaggeration ≈ 6 .

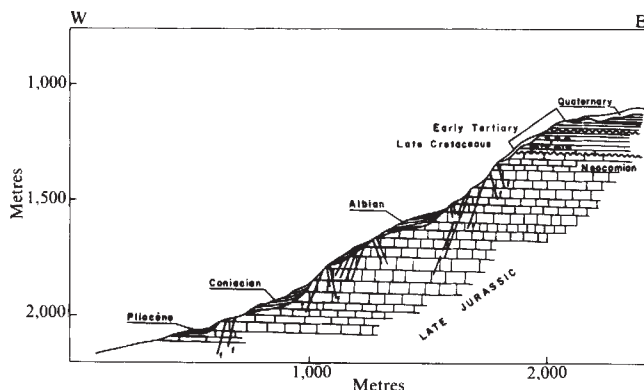


Fig. 3 Synthetic section representative of the whole observations made along the Mazagan Escarpment.

distributed within the algal pelsparitic facies. The components are often bound to grapestones similar to those of the recent shallow-water high-energy carbonate environments. (3) The pelsparites alternate with algal reef debris. The Upper Jurassic to Lower Neocomian age of the limestone was determined from dasycladacean algae. (4) Above the pelsparites the shallow environment grades into high-energy oncolitic sediments. (5) Younger sediments are represented by foraminiferal algal grain-

stones. (6) In the outer parts of the Mazagan Plateau bioclastic wackestones occur. They contain calpionellids, saccocoma and debris of siliceous sponges. The sediment was settled at the deeper foreslope of the Jurassic platform, influenced by pelagic organisms. (7) The shallow-water platform was finally covered by shallow-water oolites.

Ages range from Upper Jurassic to Lower Neocomian. The ammonite-bearing Oxfordian level⁷ could not be sampled, since it occurs only below 3,000 m depth. From middle Early Cretaceous times, thin hemipelagic nannomarls were deposited, especially on faulted blocks along the lower part of the escarpment. South of the Mazagan Plateau, along the northern branch of El Jadida Canyon around 1,200 m depth, we observed a sequence, several hundred metres thick, of well-bedded Berriasian to Barremian sandy biocalcareous clastics. Cross-lamination, slump structures and intraformational breccias suggest deposition in a slope environment. Late Aptian to early Albian semiconsolidated greenish-grey hemipelagic nannomarls were deposited only at the deeper parts of the block-faulted palaeoslope.

'Mid-Cretaceous' sedimentation is characterized by conspicuous condensation horizons, well bedded and only a few tens of metres thick. Oolitic sandstones contain ferruginous ooids; breccias (possibly Late Cretaceous to Palaeogene in age) are full of reworked clasts and fossils of different age. Late Cretaceous bio- and dolomicrites, unfossiliferous mudstones,

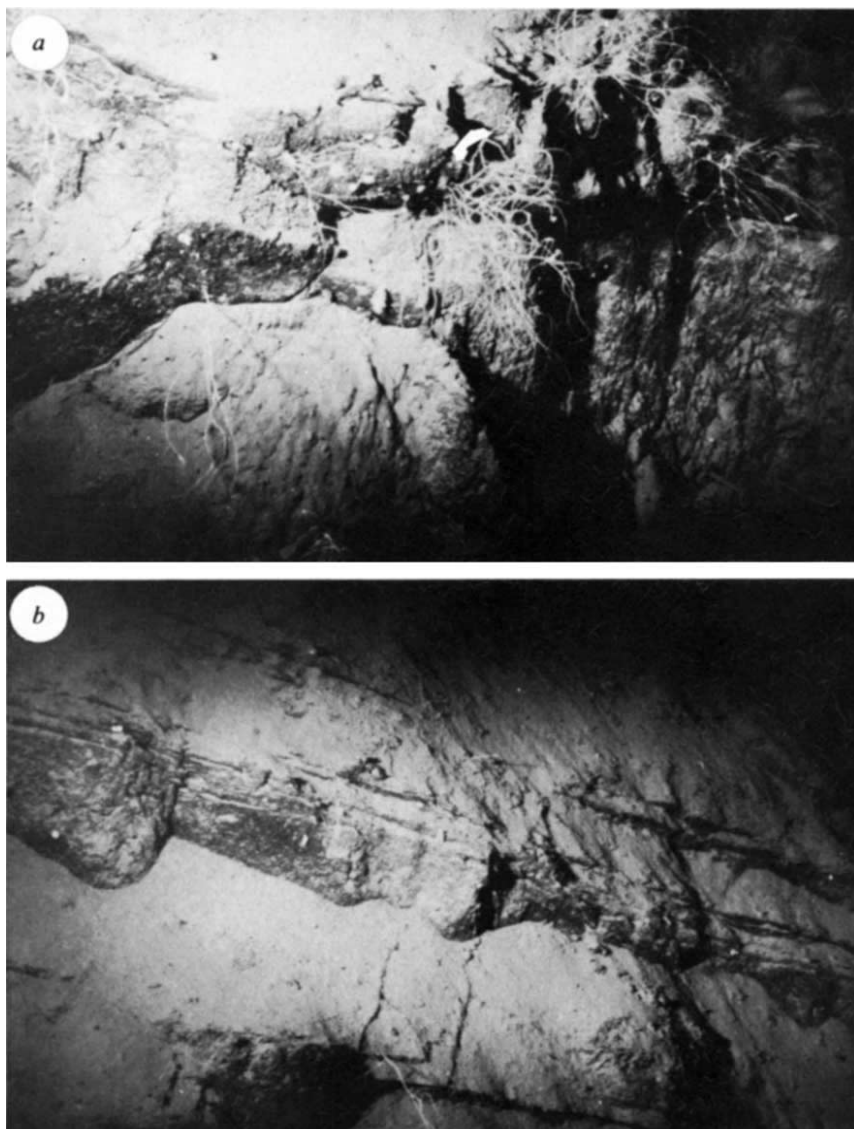


Fig. 4 *a*, Carbonate platform, dive no. 87, Upper Jurassic to Lower Neocomian limestones. On left side of the photograph a small normal fault (~50 cm high) can be seen. *b*, Bedded layers, dive no. 88, hemipelagic to pelagic marls. Late Cretaceous-Palaeogene. The approximate scale is 1:20.

dolomites, cherts and phosphatized carbonates of probable Palaeogene age overlie the platform carbonates between 1,400 and 1,000 m in the central part of the Mazagan Plateau (Fig. 4b).

From the seismic data, the Late Cretaceous–Tertiary series are up to 500 m thick on the Mazagan Plateau proper; the thickness decreases considerably towards the western edge of the plateau.

As discussed from previously studied carbonate platforms^{11,12}, the major questions of this topic are the geological evolution of the different constructional and destructional processes, such as carbonate build-up, defacing, progradation, by-pass, block-faulting, backtilting, and finally the subsidence history of this starved continental margin.

A conspicuous result of the *Cyana* dives is the lack of typical reef-build-up in the area of the present-day Mazagan Escarpment over the total vertical distance. A first explanation can be attempted: early cementation and binding of the stromatolitic pelsparites might have stabilized the platform margin; the platform foreslope environment would then be represented by the calpionellid wackestones. But from seismic evidence which shows foundered blocks seaward of the escarpment and because of the results of DSDP holes one can also imagine that the present shape of the escarpment is the result of a small retreat of the cliff due to defacing and blockfaulting.

At the beginning of the Cretaceous the subsidence rates probably increased considerably. From that period all sediments deposited on the Mazagan slope are only pelagic bathyal sediments. The observed very thin cover of well-bedded early Cretaceous nanno-oozes or nannomarl, flat-lying or lapping onto the slope, and the infilling of small pockets or dissolution pores strongly argue for downfaulting and a certain by-pass along the steep sediment-starved escarpment. The Berriasian–Barremian clastic sequence, observed in the south of the plateau, could represent the erosive remnant of a prograded delta deep-sea fan sequence.

Major evidence for erosion has been observed at the outer edge of the Mazagan Plateau. Episodic non-deposition, uplift and erosion (oolitic ironstones), reworking (breccias and thin condensed horizons), and a major mid-Cretaceous to Palaeogene hiatus can be inferred from our diving observations and seismic evidence (Fig. 2). One can imagine that the starved or partly prograded escarpment has been submitted to defacing during most of the past 100–120 Myr. Open fractures and numerous fault planes also indicate such a slope destruction. Chemical and/or biogenic dissolution could have been active (rounded surfaces of the limestones outcrops) as described from other places^{11–13}. Gravitational mass wasting is another possible mechanism with progressive spallation of the cliff¹⁴.

The individual displacement of normal faults observed during Cyamaz was only a few centimetres to decimetres. The directions of faulting have been observed *in situ* and are confirmed by seismic evidence. The main direction seems to be a N 60° trend associated with conjugate system of N 120°. These two directions have acted since the first phase of rifting (Triassic–Liassic). Another system is represented by N 20° trend essentially marked on the plateau and observed in dive 92. The last direction is the N 160°–180° which is the one of the southern part of the escarpment. Open fractures observed in the carbonate platform have not been observed in the bedded Cretaceous–Tertiary sequence above. It is not clear whether this is due to tectonic activity prior to mid-Cretaceous or to differential physical properties.

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Sm–Nd age and isotopic systematics of the bimodal suite, ancient gneiss complex, Swaziland

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Studies of the development and stabilization of the Archaean crust often focus on the relative temporal relationships between the metamorphosed basaltic to ultramafic volcanic units (greenstone belts) and the sialic gneiss terrains that make up the oldest sections of the terrestrial crust. At the heart of this interest are the questions of the processes responsible for crust formation in the Archaean and whether or not the various units of an Archaean crustal section represent new additions to the crust from the mantle or are products of the reprocessing of even older crustal materials. One area where this controversy has been particularly pronounced is the Archaean crustal section of south-west Africa^{1–6}. The oldest rocks in the Kaapvaal craton consist of the Onverwacht Group of mafic to ultramafic metavolcanics of the Barberton greenstone belt and a grey-gneiss complex termed the ancient gneiss complex (AGC) of Swaziland. We report here the results of a whole-rock Sm–Nd isotopic study of the AGC and the implications these data may have for crustal evolution in the Kaapvaal craton.

Until recently, precise geochronological information for the AGC and Onverwacht has been difficult to obtain due to post-formational metamorphism. Davies and Allsopp³, in studies of the Rb–Sr system of gneisses from the AGC, documented ages of up to 3,200 Myr to 3,300 Myr ($\lambda_{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$). However, because of the mobility of Rb and Sr during the metamorphism of these rocks, the ages determined most likely represent only lower bounds to the true emplacement age of the sequence. Based on a correlation between initial ⁸⁷Sr/⁸⁶Sr and age of a number of the individual gneiss units within the AGC, Davies and Allsopp³ suggested an emplacement age of about 3,400 Myr for the AGC parental materials. Additional whole rock Rb–Sr studies of the bimodal suite (BMS) of the AGC by Barton *et al.*⁶ suggest an even older age of $3,555 \pm 222$ Myr (2σ uncertainty), although the precision of this age does not allow its distinction from the 3,400 Myr age suggested by Davies and Allsopp³.

Early radiometric age data for the volcanic rocks of the Onverwacht Group are generally too imprecise to provide useful chronological comparisons between this group and the AGC. However, Hamilton *et al.*⁵ recently reported a Sm–Nd whole-rock age for the lower ultramafic unit of the Onverwacht Group of $3,530 \pm 50$ Myr (age and 2σ uncertainty as reported by Hamilton *et al.*⁷). The initial Nd isotopic composition determined from this isochron⁷ corresponds to $\epsilon_{Nd} = +0.8 \pm 1.1$, slightly displaced

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Table 1 Rb-Sr and Sm-Nd results

Sample	Rb*	Sr*	$^{87}\text{Rb}/^{86}\text{Sr}\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}\ddagger$	$T_{\text{BE}}\S$	Sm*	Nd*	$^{147}\text{Sm}/^{144}\text{Nd}\parallel$	$^{143}\text{Nd}/^{144}\text{Nd}\ddagger$	$T_{\text{BE}}\S$
SWZ-4	235	124	5.61	0.93098 ± 9	2,820	2.39	16.1	0.08982	0.510293 ± 18	3,310
SWZ-6	75.0	283	0.769	0.73498 ± 6	3,040	3.20	20.0	0.09661	0.510403 ± 25	3,370
SWZ-5	67.6	47.6	4.19	0.89889 ± 10	3,250	9.66	45.5	0.1282	0.511146 ± 20	3,280
SWZ-3	65.1	266	0.709	0.73013 ± 6	2,800	2.07	7.99	0.1584	0.511822 ± 24	3,090
SWZ-10	41.5	106	1.138	0.74161 ± 13	2,420	1.68	5.21	0.1953	0.512668 ± 29	—
SWZ-12	17.1	148	0.335	0.71360 ± 3	2,430	0.772	2.25	0.2078	0.512946 ± 29	4,010

* Concentrations in p.p.m. Uncertainties approximately 1% for Rb and <0.5% for Sr, Sm, and Nd.

† Uncertainty = 1%.

‡ Sr fractionation corrected to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$ and reported relative to $^{87}\text{Sr}/^{86}\text{Sr} = 0.7080$ for the E and A Sr standard. Nd fractionation corrected to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$ and reported relative to $^{143}\text{Nd}/^{144}\text{Nd} = 0.511860$ for the La Jolla Nd Standard. Uncertainties represent $2\sigma_m$ of 200–300 ratios.

§ Model ages in Myr calculated with respect to 'bulk Earth' $^{87}\text{Rb}/^{86}\text{Sr} = 0.09$, $^{87}\text{Sr}/^{86}\text{Sr} = 0.705$, $^{147}\text{Sm}/^{144}\text{Nd} = 0.1966$ and $^{143}\text{Nd}/^{144}\text{Nd} = 0.512638$ (ref. 10).

|| Determined by tracers calibrated against standard solutions prepared from Nd and Sm metal (Ames Laboratory) and cross-checked against the $n(\text{Sm}/\text{Nd})\beta$ standard²⁷. Reproducibility of tracer Sm/Nd measurements over 2 yr time is $\pm 0.06\%$.

above $\epsilon_{\text{Nd}} = 0$. $\epsilon_{\text{Nd}} = (I^{\text{SA}}/I^{\text{BE}} - 1) \times 10^4$ where I corresponds to the $^{143}\text{Nd}/^{144}\text{Nd}$ of the sample (SA) and model 'bulk Earth' reservoir (BE) calculated at the age of interest.

Because of the now-proven ability of the Sm-Nd radiometric system to provide precise chronological information for rocks that have suffered severe Rb-Sr redistribution, a Sm-Nd whole-rock and mineral isochron study of samples from the AGC was begun. Reported here are the results for whole-rock samples from the oldest unit of the AGC, the BMS¹, chosen from those whose geochemistry was studied by Hunter *et al.*⁴.

Clean surfaced rock fragments each totalling more than 200 g were powdered and divided into 100-mg aliquots for dissolution and Rb-Sr, Sm-Nd analyses. Techniques used for chemical separation and mass spectrometry have been described previously⁸. Blanks of 100 pg (10^{-12} g), 400 pg, 20 pg and 80 pg for Rb, Sr, Sm and Nd respectively, are negligible for the sample sizes used in this study.

Rb-Sr and Sm-Nd isotopic data for samples of the BMS are given in Table 1. Rb-Sr data show a considerable degree of scatter and fail to define an isochron (five out of six points lie significantly off of the best fit line). This is reflected in the wide range in Rb-Sr model ages shown in Table 1. Clearly, Rb and Sr did not remain immobile during post-emplacement alteration and metamorphism on the small, several centimetre, scale of the samples used for analysis.

In contrast to the disturbance evident in the Rb-Sr system, Sm-Nd data define precisely a line on the isochron diagram of Fig. 1. As shown in the inset to Fig. 1, all data points lie within analytical uncertainty of the best-fit line with the exception of the data for sample SWZ-6, which deviates from the line by only 5 parts in 10^5 . The age indicated by the isochron is $3,417 \pm 34$ Myr with an initial $^{143}\text{Nd}/^{144}\text{Nd} = 0.508149 \pm 31$ (age, initial, and 2σ uncertainties calculated using the 'Wendt-2' least-squares line fitting model⁹; $\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$). Using the bulk earth Sm-Nd parameters estimated from the chondritic values reported by Jacobsen and Wasserburg¹⁰ of $^{147}\text{Sm}/^{144}\text{Nd} = 0.1966$ (corrected for the difference in atomic weight of Nd resulting from the use of the different Nd fractionation correction value used at California Institute of Technology²⁷ and $^{143}\text{Nd}/^{144}\text{Nd} = 0.512638$, an initial $\epsilon_{\text{Nd}} = +1.1 \pm 0.6$ is calculated for the AGC samples. The non-chondritic initial Nd isotopic composition indicated by the isochron accounts for the scatter in the Sm-Nd model ages shown in Table 1.

The excellent colinearity of the Sm-Nd data is surprising given the wide variation in chemical composition⁴ of these samples from siliceous gneiss (SWZ-5; $\text{SiO}_2 = 76\%$) to tonalite (SWZ-6; $\text{SiO}_2 = 66\%$), diorite (SWZ-3; $\text{SiO}_2 = 57\%$), and metabasalt (SWZ-10 and 12; $\text{SiO}_2 = 49\%$). Even the data for the stratigraphically younger quartz-monzonite gneiss, SWZ-4, lie within analytical uncertainty of the best-fit line defined by the

remainder of the data. If the data for SWZ-4 are left out of the line regression, the isochron shifts to an only slightly older age of $3,450 \pm 40$ Myr with the initial ϵ_{Nd} increasing by only 0.1 unit.

Placing chronological significance on the age indicated by the isochron depends upon the validity of the assumption that all the rocks used to define the isochron initially had the same isotopic composition. For these BMS samples, with widely varied compositions, and probably differing petrogenetic histories, this assumption is particularly difficult to verify. However, the high degree of colinearity of the Sm-Nd data strongly argues for the validity of this assumption in this case. Therefore, the age of 3,420–3,450 Myr is interpreted as the time when the parental igneous rocks of the BMS gneisses were extracted from a source region that was homogeneous with respect to its Nd isotopic composition. This need not, and probably does not, mean that all of the varied chemical units used to construct the isochron were derived from exactly the same source during a single igneous event. Rather, the Sm-Nd data allow for a variety of petrogenetic mechanisms, from direct partial melting of the mantle for the basaltic components, to anatexis melting of slightly older crustal materials for the more silicic rocks, as long as these events occurred within a time interval short enough that significant Nd isotopic heterogeneity was not generated in the varied source materials of these rocks.

If the initial Nd isotopic compositions of the BMS samples were not identical at the time indicated by the isochron, the colinearity of the data requires that whatever isotopic heterogeneity existed was strongly correlated with Sm/Nd. In other words, a pseudo- or source-isochron must have existed for these samples 3,420 Myr ago. In this event, the age indicated by the isochron today would overestimate the true age of the rocks. Again, however, the high degree of colinearity of the Sm-Nd data requires a similar degree of colinearity to have existed on the 'pseudo-isochron' 3,420 Myr ago. Because of the generally large degree of scatter shown by pseudo-isochrons for recent volcanics, we consider this an unlikely interpretation of the AGC data.

Interpreted as the igneous age of the oldest rocks of the AGC, the Sm-Nd age of the BMS is distinct and approximately 100 Myr younger than that of the Onverwacht Group volcanics⁵. If the data for the stratigraphically younger SWZ-4 are left out of the line fitting routine, the age of the AGC is still some 80 Myr younger, but overlaps at the extreme limits of uncertainty, the age of the Onverwacht. However, the geological significance of the age of the BMS depends on the interpretation of exactly what is being dated by the Sm-Nd isochron. The most straightforward interpretation is that the date represents the time when the rocks of the BMS solidified from partial melts derived from the mantle and/or slightly older basaltic materials in the crust. Various alternative scenarios could be proposed

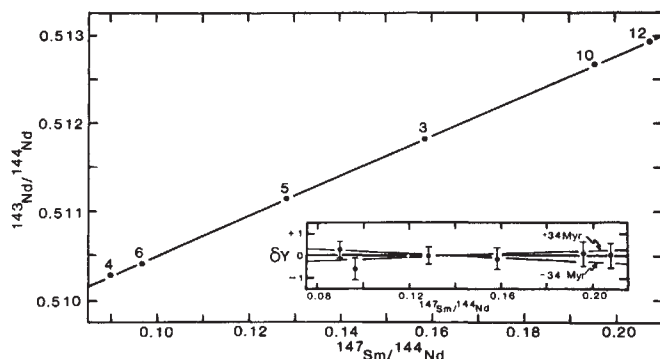


Fig. 1 Sm-Nd isochron diagram for samples of the bimodal suite. Inset shows deviations (δY) in parts in 10,000 of the data from the best fit line. The data indicate an age of $3,417 \pm 34$ Myr ($\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$) with an initial $^{143}\text{Nd}/^{144}\text{Nd} = 0.508249 \pm 31$ (all errors are quoted at the 2σ uncertainty level). This initial isotopic composition corresponds to a value of $\epsilon_{\text{Nd}} = +1.1 \pm 0.6$ (present day bulk Earth $^{143}\text{Nd}/^{144}\text{Nd} = 0.512638$, $^{147}\text{Sm}/^{144}\text{Nd} = 0.1966$)¹⁰.

that allow the basaltic members of the BMS to be considerably older than indicated by the isochron¹¹. For instance, the Sm-Nd model age of SWZ-12, with respect to a source with chondritic Nd isotopic evolution, is 4,101 Myr. However, because of the highly fractionated Sm/Nd of the siliceous BMS gneisses, the maximum age of these rocks is strictly limited by the rapid increase with age in their calculated initial ϵ_{Nd} values (1.4 ϵ_{Nd} units per 100 Myr for SWZ-4) which quickly rise to unreasonably high positive values. If the maximum value for the allowable initial ϵ_{Nd} is constrained by the initial ϵ_{Nd} determined for the mantle derived Onverwacht volcanics⁵, these light rare earth element (LREE) enriched gneisses can be no older than about 3,500 Myr. Clearly, if the most straightforward interpretation of the isochron age is correct, the AGC was produced in a distinct crustal generation event occurring some 100 Myr after the eruption of the Onverwacht volcanics. This interpretation must then be reconciled with at least two field observations of the relative ages of the AGC and Onverwacht. First, that the AGC is rather highly metamorphosed¹ while the Onverwacht is not; and second, that the AGC stratigraphically underlies¹² a series of metamorphosed mafic volcanics (the Dwalile metamorphic suite) that are chemically similar to the Onverwacht volcanics.

Regardless of the relative ages of the Onverwacht and the AGC, their positive initial ϵ_{Nd} values show that they represent new additions to the crust from the mantle. Neither appears to be the product of reworking of a much older (>50 Myr older), LREE enriched, sialic crustal section. Furthermore, the positive initial ϵ_{Nd} of these rocks implies that their mantle source materials had been relatively depleted in LREEs, and presumably other incompatible elements, by a much older episode of melt removal. The increasing occurrence of positive initial ϵ_{Nd} for old crustal rocks^{5,7,13-15} (Fig. 2) suggests that either a chondritic model for the Nd isotopic evolution of the Earth's mantle is inappropriate or that the mantle was chemically heterogeneous for a considerable time before the formation of the oldest crustal sections.

One class of models predicting heterogeneities in the early Earth's mantle calls upon ancient (>4,000 Myr) differentiation through the formation of proto-continental crusts^{16,17} and/or a major molten episode in the Earth's infancy^{18,19}. A major difficulty with this category of model is that though evidence for ancient mantle reservoirs depleted in incompatible elements is available, similar evidence for correspondingly enriched reservoirs is lacking in the crustal geological record^{7,20}. This seems particularly surprising for two reasons. First, most models for an early terrestrial differentiation place this 'enriched' reservoir in a proto-crust that is sufficiently buoyant to resist subduction,

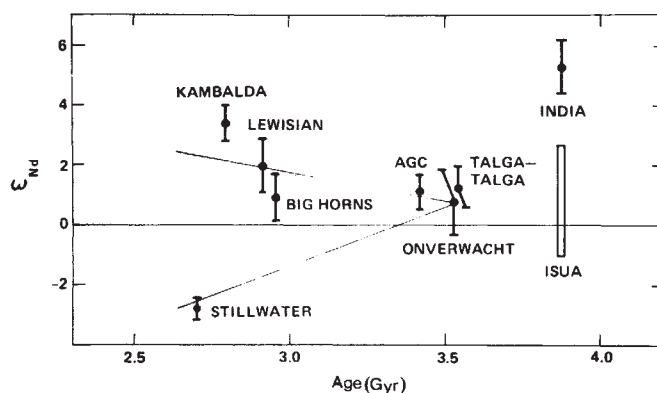


Fig. 2 Nd isotopic evolution diagram for ancient crustal rocks for which initial Nd isotopic compositions have been determined by Sm-Nd isochrons. Initial Nd isotopic compositions of Isua⁷, India¹³, Talga-Talga²⁴, Onverwacht⁵, Lewisian²⁵, Big Horns¹⁵, and Kambalda¹⁴ plot slightly above $\epsilon_{\text{Nd}} = 0$ suggesting a source for these rocks relatively depleted in LREE compared to chondrites. Only the data for the Stillwater intrusion²⁶ plot below $\epsilon_{\text{Nd}} = 0$ which may imply contamination of the Stillwater primary magma by older crustal material²⁶. Lines originating from the initial ϵ_{Nd} of the Onverwacht Group show the evolution of Nd isotopic composition in the basaltic members of these volcanics given their measured Sm/Nd ratios⁵.

and rehomogenization, into the mantle¹⁹; second, a reservoir depleted in the LREE would also be expected to be depleted in other incompatible elements, especially the radioactive heat producing elements K, U, and Th. Continued melt production over Earth history from a depleted reservoir with relatively limited internal heat production rather than a more obvious expression of melting of the correspondingly enriched source is not easily explained.

Another stumbling block for any model calling on either a non-chondritic Earth or a very early (>4,000 Myr) terrestrial differentiation event is the lack of imprint of such an event in the source of modern volcanics. Young, unquestionably mantle derived, oceanic volcanics give source U-Pb²¹, Rb-Sr²² and Sm-Nd²³ model ages of only 1,000–2,000 Myr. To some extent this problem can be overcome by assuming that convective exchange between differentiated reservoirs in the crust and mantle has imperfectly remixed the fractionated reservoirs created over Earth history. In this case, the model ages obtained for rocks derived from these mixed reservoirs would have little, if any, time significance. Because of the possible influence of convective mixing in the mantle in masking the effects of differentiation events occurring during early Earth history, further constraints on our understanding of the early geochemical evolution of the Earth clearly must be sought in more complete and precise isotopic data for the ancient rocks of the continental crust.

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Epigenesis of cowbird song—A joint endeavour of males and females

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The role of social stimulation from female cowbirds (*Molothrus ater*) on male song learning was tested. In this species the females do not sing. However, we have found that both male and female cowbirds contribute to the maintenance of geographic variation in the songs of the two subspecies, *Molothrus ater* (AT) and *Molothrus ater obscurus* (OB)^{1,2}. We therefore performed two studies using males of the eastern subspecies (AT) reared in complete isolation from male conspecifics. In the first study, AT males, tutored with AT song, were individually housed with other species or with females of the southern *M. a. obscurus* subspecies (OB). In the second study, naive AT males were housed, without tutoring, with other species or AT or OB females. In both studies the males developed significantly different song repertoires that were biased towards the preferences of their non-singing female companions. These data establish a new, non-auditory, source of vocal instruction: social stimulation from the song's intended recipient.

Our earlier results revealed reciprocal contributions by male and female cowbirds to the maintenance of geographical variation in the songs of the two subspecies, AT and OB (Fig. 1). Juvenile AT males became bilingual (singing clear renditions of both AT and OB variants) when exposed to OB males¹. Adult AT males did not learn OB song when housed with OB males, but they did do so when housed with OB males and females². Moreover, adult AT and OB males substantially altered their songs when housed only with non-singing females of other subspecies (A. P. K. and M. J. W., unpublished results). The preferences of females for their native geographic variant were found to be highly resistant to postnatal modification^{3,4}. Song biases were also evident in choice of mates in that captive females copulated most often with males who sang the highest proportion of the female-appropriate song variant².

In this study we searched for ontogenetically prior instances of mutual adaptation. Could the female affect song outcome not only by her choice of mates but also by her presence during development itself?

The AT males were hand-reared from 4–5 days after hatching in acoustic isolation until, at 50 days old, they were placed in their respective conditions using previously described procedures³. All females were adults: the AT birds were caught in the wild in Orange County, North Carolina and the OB birds in Starr County, Texas. Non-conspecific companions were male and female canaries (*Serinus canaria*) for all males except one

Table 1 Effects of housing on song development of males tutored with AT song

	Males housed with other species			Males housed with OB females		
	RDB	2B	DBW	RLB	RW	P
Song content						
% Full tutored song	24	34	21	0*	58	0
% Partial tutored song	48	66	38	30	0	0
Original song	28	0	41	70	42	100
No. of original song types	2	0	1	3	3	3

A song was defined as copied if it possessed the same sequence of frequency contours of the same frequency duration and with the same minimum and maximum frequency (see Fig. 1). Full tutored song constituted copies of the tutored AT song; partial songs were copies of the AT song that consisted of only the first song phrase; original songs were any non-copied song; and song types were stereotyped variations of original themes which could be AT or OB. The group differences for full and partial tutored song and the number of song types were significant as tested by the Mann-Whitney test ($U=0$, $P<0.05$ for all comparisons).

* Male RLB sang the tutored song during the winter but deleted it from his repertoire in April.

Table 2 Effects of housing on non-tutored males

	Males housed with:		
	Other species	AT females	OB females
Mean value			
Frequency width of phrase 1 (Hz)	3,517 (1,033–5,903)	3,091 (2,100–4,591)	5,912 (5,210–6,800)
No. of notes in phrase 1	5.2 (3–13)	4.8 (2–7)	2.6 (1–4)
% AT song	26 (16–37)	51 (18–93)	15 (0–38)
% OB song	36 (0–63)	0 (0)	64 (2–100)
% Partial song	38 (0–84)	49 (0–98)	21 (0–50)

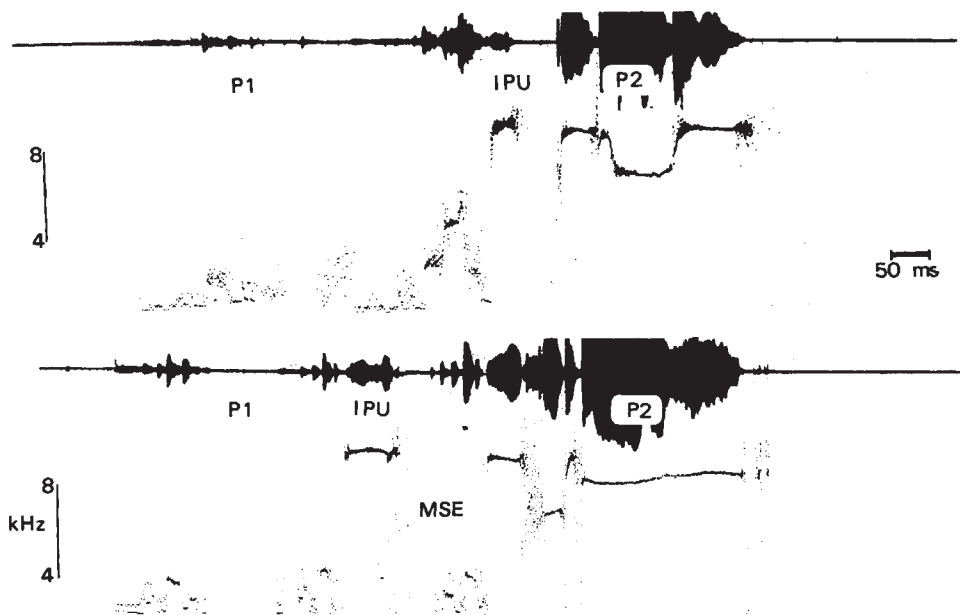
Frequency range refers to the bandwidth (in Hz) of the maximum frequency modulation of the notes in phrase 1; no. of notes is the number of discrete frequency modulations of phrase 1; % AT or OB refers to the percentage of songs classified as AT and OB on the basis of the presence or absence of the MSE (see Fig. 1); partial song refers to songs sung without an inter-phrase unit or composed only of whistles. Values in parentheses are the range. Kruskal Wallis' one-way analyses of variance were significant at d.f. = 2 and $P<0.049$ for: frequency range ($H=5.9$), no. of notes ($H=6.8$), % AT ($H=6.5$) and % OB ($H=6.7$). Mann-Whitney U -tests were used to compare the mean differences of males reared with AT versus OB females and all were significant: frequency range ($U=0$, $P<0.008$), no. of notes ($U=0$, $P<0.008$), % AT ($U=1$, $P<0.016$), and % OB ($U=0$, $P<0.008$).

which was housed with a female starling (*Sturnus vulgaris*). Methods of housing and recording followed techniques described elsewhere¹.

In the first study, six naive AT males were tutored daily (240 repetitions of the same AT song over 3 h) for 3 months (September to November). Three males were separately housed with one or two birds of other species and the remaining three were housed individually each with two OB females. In the second study, 12 naive AT males were housed separately: three with one or two individuals of other species, five with 2–4 AT and four with two or three OB females.

During both experiments, singing behaviour was monitored from November to June. In all, 23,000 songs were recorded from males and none from females. The only vocalization made frequently by the females was a call given during feeding, usually transcribed as a 'chuck' or 'kek' sound. This call is made equally frequently by the males. In addition, females produced 'rattle' vocalizations in May and June. These calls were acoustically the

Fig. 1 Zero-crossings analyser display of typical *M.a. ater* (AT, upper panel) and *M.a. obscurus* song (OB, lower panel). P1 represents the first phrase; IPU, the inter-phrase unit; MSE, the mid-song element; and P2, the whistle phrase. The MSE is found only in OB song¹. Playbacks of songs where the presence or absence of the MSE was manipulated demonstrated the sensitivity of OB, but not AT, females to this element: when the MSE was removed, OB, but not AT, females showed a significant decrease in the copulation response to OB song⁷. Thus, the operational definition of AT song is P1 IPU P2 and that of OB song is P1 IPU MSE P2. Cowbird song typically ranges from 500 to 12,000 Hz.



same in AT and OB females. Female cowbirds have never been reported to sing in the wild nor have we observed it in 10 years of laboratory recording⁵.

The data reported here are based only on songs recorded when the males were in full breeding condition as determined by courtship activity. The songs of each male were recorded during two separate sessions on at least 3 days for a minimum of 7 recording hours; 1,036 songs were analysed in the first experiment and 2,191 in the second. The mean numbers of songs sampled for males in each condition were comparable. In the first experiment, a mean of 165 songs (range 133–213) was obtained for the males housed with other species and 180 (range 114–226) for the males housed with OB females; in the second experiment, an average of 175 songs (range 115–252) was analysed for the males housed with AT females, 188 (range 95–247) for the males housed with OB females and 188 (range 165–205) for males housed with other species. We based our sampling criteria on other data comparing the daily song samples of 14 males recorded for a 7-week period, with over 500 songs from each male. These data indicated that 95% of a male's song types (males have 1–6 song types) are represented in 87% of daily samples of 11–20 songs, 98% are represented in samples of 21–40 songs, and 99% in samples of 41–85 songs (D. H. Eastzer, unpublished observations).

The standardized auditory experience provided for the males in the first study produced significantly different outcomes (Table 1). Copied song (clear renditions of the tutor song) constituted 77% of songs of males housed with other species and only 29% of the songs of males housed with OB females. Conversely, the males housed with other species sang an average of 23% original songs while the males with OB companions sang a mean of 71% original songs. Males housed with other species also produced fewer stereotyped versions (song types) of original song while males housed with OB females produced more original song types. Finally, one of the AT males housed with OB females developed an OB song type. This finding contrasts with previous data from nine juvenile AT males caught in the wild and housed individually with AT females: in that case, no males produced an OB song although they too were limited in their early exposure to AT song².

The second study afforded another view of the potential influence of the female because auditory stimulation was not provided; the impact of the female was even more conspicuous. Due to the absence of a standardized song input, song development was measured differently. Based on previous data showing that the first half of cowbird song accounted for over 90% of song potency, acoustic differences in it were compared across the three groups^{6,7}. In addition, the proportion of each male's

songs that was prototypically AT or OB was computed (Table 2). Significant differences occurred on all measures, most notably between males housed with AT versus OB females. The performance of the males housed with other species was almost uniformly intermediate, highlighting the 'biasing' effect of female presence.

Thus, the presence of females affected the male's tendency to incorporate auditory input and his organization of auditory output. As such, these data go beyond investigations of other species in which the social properties of vocal transmission have been identified⁸. In that case, the social transfer of information was vocal, that is, the 'tutor' sang and the 'pupil' listened and copied what he heard. In the present study, as the female cowbirds never sang, the transmission of information did not rely on modelling or imitation.

Although cowbirds are brood parasites, and hence restricted in their access to early species-typical stimulation, they seem to rely on learning as much as other non-parasitic species⁹. As the males reared with other species appeared to possess geographically nonspecific repertoires, the role of the female may be to guide the male through trial and error learning towards an adaptive repertoire.

There have been sufficient observations of potential social influences in other species to suggest that the processes described here are of broad significance^{10–15}. The concept of the social mediation of song learning is not inconsistent with present formulations on the nature of the acoustic controls¹⁴. Rather it points to a new and socially derived origin for some of the constraints on vocal learning. As such, the data also address the parallels between song and speech: songbirds, like humans, may need to experience not only the sounds but the consequences of their vocal endeavours.

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Sexual selection in a hermaphroditic plant

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The ornaments and weapons of male animals are among the showiest and seemingly most wasteful of nature's productions. Darwin's theory of sexual selection showed how such traits could be selected through competition for mates even if they were otherwise detrimental¹. Flowering displays of plants often show a comparable degree of gaudiness and profligacy, but exploration of the role of sexual selection in plants has only just begun²⁻⁵. Bateman⁶ argued that sexual selection is caused by the greater ability of males than females to increase fitness by mating repeatedly, due to the females' greater energetic commitment to gametes or parental care. Similar reasoning applies to hermaphrodites^{2,3}. In hermaphroditic milkweeds, most young fruits are aborted⁷⁻¹⁰ and female reproduction (seeds) is limited more by resources than by pollination¹¹. Sexual selection theory therefore predicts that traits increasing mating success will have evolved because they increase male success through pollen. Here I report that a suite of floral traits of a hermaphroditic plant is best interpreted as having evolved through the male competition component of sexual selection, a result with important implications for evolutionary studies of pollination systems.

I studied *Asclepias exaltata*, a herbaceous, perennial milkweed, in southeastern Michigan. Each hermaphroditic flower has five pollinaria, each of which consists of two pollinia composed of about 180 fused pollen grains. The intricate pollination mechanism is like that of other milkweeds¹². A fissure subtends each pollinarium and the pollinarium is removed when an insect withdraws a leg or other appendage that has slipped into the fissure. The pollinarium, now attached to the insect, dries into an orientation that facilitates the next step¹²⁻¹⁴; if the insect draws the same appendage past another fissure, a pollinium may detach and remain in the fissure, and fertilization follows. Self-pollination is possible but pollinia are usually carried far enough to prevent it (see below).

I estimated the number of pollinaria removed from each umbel (inflorescence) from counts on five randomly chosen flowers. The umbel estimates were summed for each plant to obtain an estimate of relative male success. Female fitness was estimated by counting fruits (seed counts were discontinued when I found that plant fruit number was highly correlated with seed number— $r=0.95$, $n=111$ —and that seed number per fruit did not change when fruit numbers were experimentally reduced).

To simulate the fitness effects of producing fewer flowers, I removed half the flowers from each umbel on randomly chosen plants. In another population, I simulated decreased flower longevity by bagging all umbels of some plants (thereby excluding pollinators) for 4 days of the 7-9-day anthesis period. Table 1 compares these experimental plants with their untreated control groups. Plants in both experimental groups had reduced pollinarium removals but, despite initiating fewer fruits, did not mature fewer.

Thus, plants can raise a full complement of fruits with half their flowers or with flowers open half as long. Unless the male

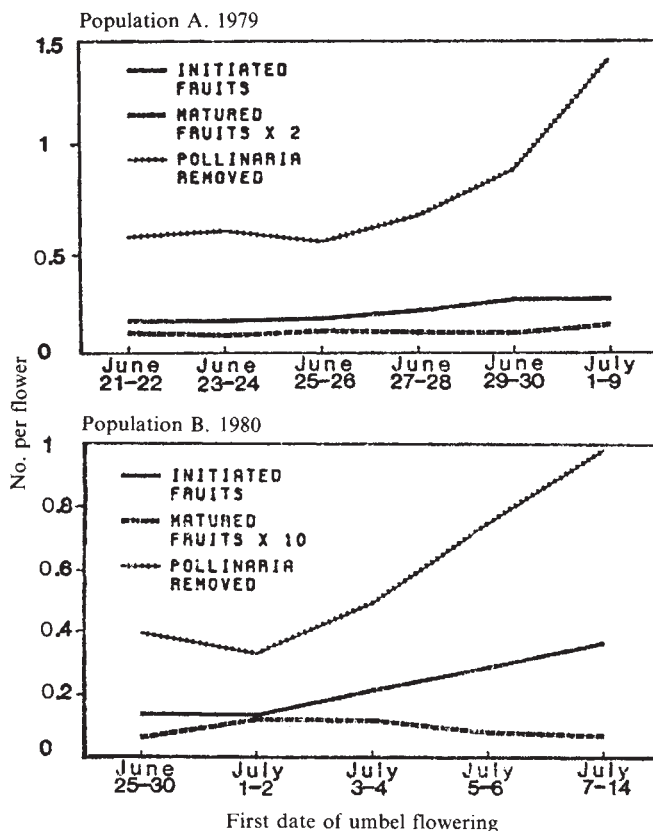


Fig. 1 Seasonal changes in per flower reproductive measures. Pollinarium removal rates vary seasonally, as do fruit initiation rates ($P<0.0001$ for each in both populations, Kruskal-Wallis test), but fruit maturation rates do not (Kruskal-Wallis or χ^2 for presence of mature fruit). Seeds per flower, measured only in population A and not shown, did not vary either. Numbers of umbels for each period in population A are 69, 71, 70, 60, 76, 25, and for population B, 48, 56, 87, 54, 25. Some dates were grouped to increase sample size.

fitness measure is badly biased, it appears that flower numbers and longevity are maintained to enhance male competition. Because about 95% of pollinator visits were by bumblebees, it is unlikely that such a bias could arise through differences between treatments in pollinators. A bias could exist if there are strongly diminishing returns on pollinarium removals due to saturation of neighbouring plants⁴, but pollinia are probably dispersed far enough to avoid this. The time required for pollinaria to dry into the orientation most suitable for insertion (107 ± 58 s, $n=40$) far exceeds the time pollinators remain on one plant (31.3 ± 29.6 s, $n=105$). Moreover, *Asclepias syriaca* pollinia have been shown to remain on bumblebees for an average of several hours^{15,16}.

A possible alternative is that such traits are selected because, by increasing fruit initiations, they allow choice of superior fruits¹⁷. Although it seems unlikely that any such gain would be as large as the male benefit, the above experiments cannot exclude it. However, it can be excluded for a third 'male' trait, the seasonal distribution of flowers. In terms of male competition, the best time to produce a flower is whenever other flowers produced at the same time have the highest probability of maturing fruit (that is, less pollen competing for more fruit). Selection on the male function should therefore lead to flowers being shifted from times when small fractions of flowers mature fruits to periods of high maturation probabilities. This selection should persist until the fruit/flower ratio is constant throughout the flowering season, at which point no flower gains a male advantage due to its timing. This model makes some assumptions that will be detailed elsewhere. A critical assumption, that early flowers gain no large advantage by pollinating later ones (the

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Table 1 Plant reproductive measures for experimental treatments and controls

	E1	C1	<i>p</i>	E2	C2	<i>p</i>
Pollinaria removed	6.36 (50)	12.45 (89)	0.01	18.4 (33)	33.5 (112)	0.03
Fruits initiated	3.44 (50)	5.01 (137)	0.06	5.39 (33)	9.02 (112)	0.02
Fruits matured	0.320 (50)	0.234 (137)	—	1.67 (33)	2.23 (112)	0.18

Treatments are: E1, half the flowers removed from each umbel; E2, pollinators excluded from each umbel for 4 days; C1 and C2 are the corresponding untreated controls. Sample sizes are in parentheses. Significance levels are for one-tailed Mann-Whitney *U*-tests, so no level is reported when the observed difference is opposite to the hypothesized one.

converse being impossible), is probably valid because the ratio of fruit initiations to pollinarium removals does not increase during the season.

In this model, selection on the female function may determine when fruits are produced, but selection on the male function alone causes flowering to be timed so as to keep the fruit/flower ratio constant. In a species with excess pollinations, like *A. exaltata*, no single flower distribution maximizes fruit number because a plant could shift many of its flowers and still be adequately pollinated. If, on the other hand, there is selection to maximize choice of high-quality fruits, flowers should be concentrated at times when the largest fraction is pollinated.

The data in Fig. 1 confirm the prediction for selection on the male function only. There is no seasonal change in fruits per flower, despite an increase in pollination levels as measured by either pollinarium removals or fruit initiations. The observed pattern is not due to plants spacing out the drain of fruiting on resources because all fruits achieve most of their growth after the end of flowering. The pattern is inconsistent with maximizing choice because few flowers are produced at the best time for pollination. Choice might occur, but it is not important enough to alter the optimal male strategy.

These results suggest that flower number, umbel longevity and the seasonal distribution of flowers are traits selected primarily for their effects on male competition. In other milkweeds, flower number per umbel is another such trait⁸⁻¹⁰. With apologies to Charles Baudelaire, I call this the fleurs-du-mâle syndrome. Since seed production is often resource-limited in plants¹⁸, the fleurs-du-mâle syndrome should be common; traits increasing pollination levels should often be primarily male traits. An important consequence is that the usual measure of fitness in plants, seed production, will often be inappropriate for evolutionary studies of pollination systems.

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Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo

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The widespread use of clomiphene citrate and exogenous gonadotrophins for *in vitro* fertilization (IVF) in humans^{1,2} frequently results in the production of multiple embryos. Replacement of more than two embryos increases pregnancy rate³ but may result in multiple pregnancies with increased pre- and post-natal abnormality. Preservation of embryos for a limited time allows fewer embryos to be replaced on several different occasions and thus the problems of multiple pregnancy can be minimized, the effectiveness of a single IVF procedure increased and embryo replacement in adverse maternal conditions avoided. Preimplantation embryos have been successfully cryopreserved in many animal species. The sensitivity of embryos to cooling and freezing varies between species and stages of embryo development⁴⁻⁶. We report here the cryopreservation procedures that allow a high survival rate of four- and eight-cell human embryos and the establishment of a pregnancy following the freezing and storage of an eight-cell embryo for 4 months in liquid nitrogen. The pregnancy terminated at 24 weeks' gestation due to development of a septic *Streptomyces agalactiae* chorion amnionitis after premature membrane rupture.

Embryos were provided by patients participating in IVF and who had requested embryo preservation. Four- and eight-cell embryos (34-60 h culture *in vitro*) were frozen using either glycerol or dimethyl sulphoxide (DMSO) as cryoprotectant and subsequently thawed at the appropriate time following spontaneous ovulation in the patient. Embryos were transferred to the patient only if at least 50% of the original blastomeres appeared morphologically normal after 4-12 h culture following thawing and removal of the cryoprotectant. A total of 16 embryos were transferred to 15 patients.

Both four- and eight-cell embryos can survive with the majority of cells intact, using a variety of procedures (Table 1). Greater success was achieved using DMSO as cryoprotectant. Early-cleavage-stage human embryos, unlike morulae and blastocysts, equilibrate very slowly in glycerol solution because of the low permeability of glycerol⁷, and low intracellular levels of glycerol may reduce embryo survival, particularly if embryos are cooled slowly to low temperatures. Moreover, it is difficult to remove glycerol after thawing and we have frequently observed rapid hydration of cells during removal of the cryoprotectant at concentrations of glycerol below 0.33 M and excessive swelling of blastomeres. If the embryos are placed in isotonic sucrose this rapid hydration does not occur. We have yet to establish complete embryo viability by establishment of pregnancy after freezing in glycerol, although embryo survival appears to be high when four-cell embryos are frozen by the short technique and thawed rapidly (Table 1). DMSO is more permeable than glycerol and appears to be a suitable cryoprotectant for both rapid and slow thawing (Table 1). All embryos which appeared morphologically normal were replaced *in utero*. No further assessment of these embryos was permitted under the ethical guidelines established for this work.

Of the 15 patients receiving embryos, pregnancy was established in one (Fig. 1). The embryo was one of four obtained from a patient who had suffered from primary infertility for 12 yr because of bilateral tubal blockage. The details of her initial treatment for IVF are given in Fig. 2 legend. Following transfer of three four-cell embryos, pregnancy was initiated but

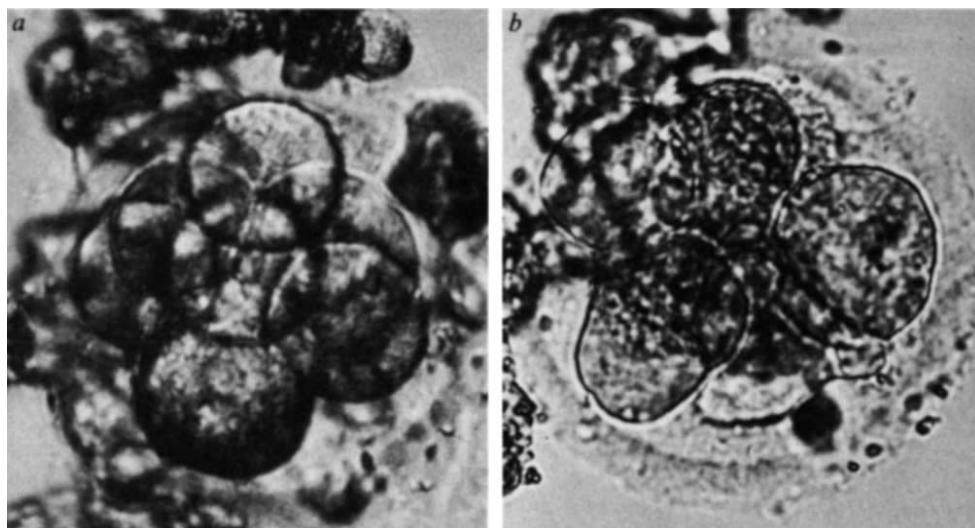


Fig. 1 Micrographs of the embryo which resulted in pregnancy before freezing (a) and immediately after thawing and DMSO removal (b). The embryo was frozen in 1.5 M DMSO phosphate-buffered saline¹² supplemented with 15% fetal calf serum, then cooled from -6°C to -80°C at 0.3 min^{-1} before storage in liquid nitrogen. The embryo was thawed at $10^{\circ}\text{C min}^{-1}$ from -120°C to 0°C . The contracted and distorted appearance of the blastomeres following thawing and DMSO removal is clearly visible (b). At this time five blastomeres appeared to have survived thawing. After culture *in vitro* for 12 h the five surviving blastomeres returned to their original size and appearance.

the patient miscarried 8 weeks after transfer. Four months later the couple requested the replacement of their frozen embryo. Hormone levels were followed during a spontaneous ovulatory cycle to detect the onset of her luteinizing hormone (LH) surge (Fig. 3). The patient was admitted to hospital after the embryo was thawed and the embryo was transferred 94 h after the start of her urinary LH surge.

The establishment of pregnancy was confirmed by normal levels of human β -chorionic gonadotrophin, oestradiol-17 β and progesterone over the following 19 weeks and ultrasonic scanning at 7, 12 and 18 weeks. Fetal growth rate and appearance at ultrasound were normal. A single episode of heavy bleeding occurred on the day of the first ultrasound (7 weeks) but this ceased the next day. The patient was given 25 mg progesterone intramuscularly (Proluton, Schering) daily for 5 weeks which is routine for such cases.

Table 1 Proportion of cells of four-cell and eight-cell human embryos surviving slow or rapid thawing after freezing in glycerol or DMSO

Cell stage	No. of embryos	Cell survival			
		Glycerol cryoprotectant		DMSO cryoprotectant	
		Rapid thawing	Slow thawing	Rapid thawing	Slow thawing
4	11	4/4 (4) 2/4 (2)	2/4 (1) 0/4 (2)	0/4 (1)	2/4 (1)
8	10	5/8 (1) 0/8 (1)		7/8 (1) 6/8 (1)	8/8 (1) 6/8 (1) 5/8 (2) 4/8 (1) 1/8 (1)

The number of cells surviving after thawing in each embryo is expressed as a fraction of the cells present in the embryo before freezing with number of embryos observed in parentheses. Embryos to be frozen were examined and photographed at $\times 100$ magnification and frozen in clean sterile glass ampoules containing 0.2–0.4 ml 1 M glycerol or 1.5 M DMSO. The addition of the cryoprotectant involved 10-min stepwise incubations at room temperatures in 0.17, 0.33, 0.67 and 1.0 M glycerol solutions or 0.25, 0.5, 1.0 and 1.5 M DMSO solutions. The cryoprotectant was removed in a similar manner with the insertion of additional steps at concentrations of 0.75 and 1.25 M DMSO and 0.33 and 0.83 M glycerol. Cooling and freezing were carried out in a biological freezer. The ampoules were cooled from room temperature to -6°C at $2^{\circ}\text{C min}^{-1}$ and ice nucleation induced at -6°C by touching the ampoule with cold forceps. The ampoules were held at -6°C for 20–30 min after seeding and then cooled at a rate of $0.3^{\circ}\text{C min}^{-1}$ to either -39°C (short technique) or to between -60°C and -80°C before transfer to liquid nitrogen. All embryos transferred to liquid nitrogen from -39°C were thawed rapidly by immediate transfer from liquid nitrogen to a water bath at 30°C . All embryos transferred to liquid nitrogen after cooling to below -60°C were thawed at $10^{\circ}\text{C min}^{-1}$ from -80°C to 4°C .

The pregnancy proceeded uneventfully until 23 weeks when the patient reported premature rupture of the amniotic membranes. The liquor was clear, uterine activity was normal and a positive fetal heartbeat detected. Four days later vaginal swabs showed heavy contamination of *S. agalactiae*. The patient was given antibiotics (250 mg ampicillin every 6 h). After the sixth day a purulent discharge was detected and the patient developed a fever. Intravenous penicillin was given and a positive fetal heartbeat continued. On the eighth day no fetal heartbeat could be detected, the patient was induced to begin labour and a stillborn female child delivered.

Pathological investigations showed the complete absence of any malformations macroscopically and histologically. The cause of death was a result of severe chorion amnionitis, funisitis and villitis with bilateral aspiration and intrauterine pneumonia due to a severe infection of *S. agalactiae* (group B). Attempts to

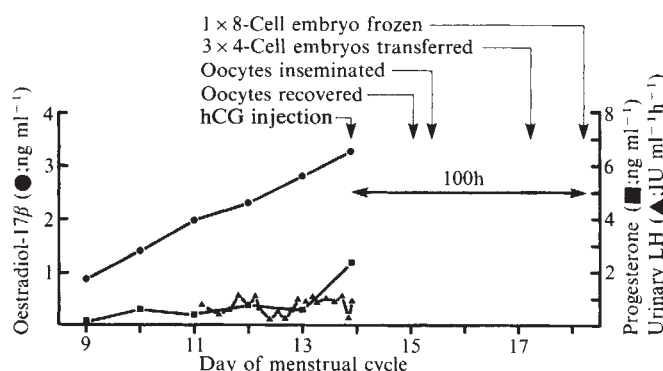


Fig. 2 For the collection of oocytes, the patient was given 150 mg clomiphene citrate (Clomid; Merrell) on each of days 5–9 of the menstrual cycle and 75 IU human menopausal gonadotrophin (Humagon; Organon) on day 10. Blood was taken daily (08.00 h) and assayed for oestradiol-17 β and progesterone on days 9–14. Urine was collected every 3 h from days 11–14 and assayed for LH excretion rate¹³. No spontaneous LH surge was detected in urine but plasma progesterone concentration significantly increased at 04.00 h on day 14 just before administration of 5,000 IU human chorionic gonadotrophin (hCG; Pergonal; Organon). Oestradiol-17 β concentration in peripheral plasma had shown a steady increase from days 9 to 14. Four mature oocytes were recovered from the aspiration of five large follicles at laparoscopy, 30 h after giving hCG¹⁴. The oocytes were preincubated for 6 h in 1 ml T6 culture medium containing 10% patients serum in 5 ml Falcon culture tubes (Falcon Plastics) before insemination with approximately 63×10^3 spermatozoa prepared from the husband's semen sample³. Two pronuclei were visible in all four oocytes 16 h after insemination and at 43 h three four-cell embryos were transferred to the patients' uterine cavity¹⁵. The remaining embryo was frozen at the eight-cell stage 64 h after insemination.

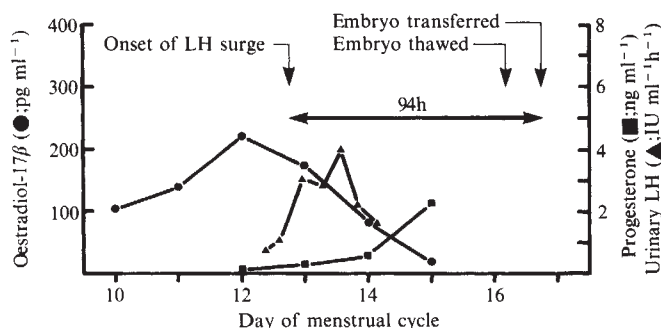


Fig. 3 Blood was obtained on days 10–15 of the menstrual cycle and assayed for oestradiol-17 β and progesterone. Urine was collected every 6 h on days 12–14. The onset of the spontaneous LH surge was considered to have occurred at about 02.00 h on day 13. Progesterone concentration showed a significant rise at 08.00 h on day 15 confirming ovulation. The frozen embryo was thawed 81 h after the onset of the LH surge, cultured in 1 ml T6 culture medium containing 10% fetal calf serum for 12 h and then transferred to the patient's womb.

culture blood and tissues for cytogenetic analysis were unsuccessful. There appeared to be no abnormality of the fetus at week 24 which could be associated with cryopreservation of the embryo.

The embryo was thawed 13 h before transfer and cultured for 12 h to allow recovery of cell function and a reassessment of cryoinjury. Implantation and development of a frozen 8-cell embryo despite damage to three of the blastomeres is consistent with observations in other species that the total complement of blastomeres in preimplantation embryos is not necessary for development to term. Isolated blastomeres or pairs of blastomeres separated from embryos up to the eight-cell stage may develop normally to live young^{8,9}. The freeze-thaw technique described here may not be the optimum, although the majority of eight-cell embryos frozen in this way had 50% or more morphologically normal cells after thawing and culture (Table 1). Blastomeres of preimplantation embryos are commonly damaged after freezing and thawing but there are no indications that live-born abnormalities are increased following embryo preservation¹⁰.

There are many new implications of human embryo preservation. Our own views on the ethics of embryo freezing have been stated previously¹¹. Guidelines established by the Australian National Health and Medical Research Council limit storage of human embryos to less than 10 yr although it may be technically feasible to store them longer. If the survival rate of human embryos approaches that for other species, embryo freezing will increase the pregnancy rate and the number of pregnancies from a single collection of multiple oocytes, increase the efficiency of IVF, and reduce the cost and the risks of repeated IVF treatment and laparoscopy. IVF and embryo freezing could be appropriate when conditions are diagnosed which may lead to permanent sterility, such as endometriosis or pelvic infection or before radio- or chemotherapy. Embryo preservation could aid the detection of genetic abnormalities through the provision of time to allow detection of genetic faults. Cells could be separated from embryos for assessment and the embryos frozen to be thawed and replaced when normality was confirmed.

Ethical difficulties may arise if the parents disagree as to the destination of the stored embryos and at the present time this would require settlement by the processes of law. Patients may be asked to include in their will their preferences concerning the destination of embryos, in case of the death of one or both parents. When situations arise where patients are unable to have their embryos replaced, their options may include the donation of embryos to couples where both the husband and wife are sterile, or it may be considered that the patients have the right to dispose of the embryos. These matters are being considered at present by Australian Law Reform Commissions.

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Note added in proof: Using the same method of cryopreservation, a second pregnancy is progressing normally at 12 weeks' gestation in a patient with tubal occlusion.

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The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage

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Toxic shock syndrome (TSS) is a complex of generalized symptoms caused by a local staphylococcal infection, and a circulating toxin is thought to be involved. Indeed, nearly 100% of TSS isolates produce an exoprotein, TSSE, that is thought to have an aetiological role on the basis of positive animal tests (refs 1, 2 and F. Quimby, personal communication) and human serological data³. Although the precise role of TSSE in TSS remains unclear (E. Kass, personal communication), no other staphylococcal factor has been implicated. Our preliminary studies of the genetics of TSSE production failed to demonstrate plasmid or phage involvement or linkage with known chromosomal genes (ref. 4 and B.N.K. *et al.*, unpublished data); however, Schutzer *et al.* have found that most TSS strains harbour prophages with common plating characteristics and suggest that the toxin(s) involved in TSS are transmitted by lysogenic conversion⁵. We show here that TSSE is not demonstrably transferred by lysogeny; moreover, we have cloned the gene and found that the cloned product is serologically and biologically indistinguishable from the native protein, and that the TSSE determinant is associated with a larger DNA segment that is absent or rearranged in TSSE⁻ strains.

In view of the apparent difference between our unpublished observations and the results of Schutzer *et al.*⁵, we analysed an additional 20 independently isolated TSSE-producing strains for lysogeny and for the ability to transfer TSSE by lysogenic

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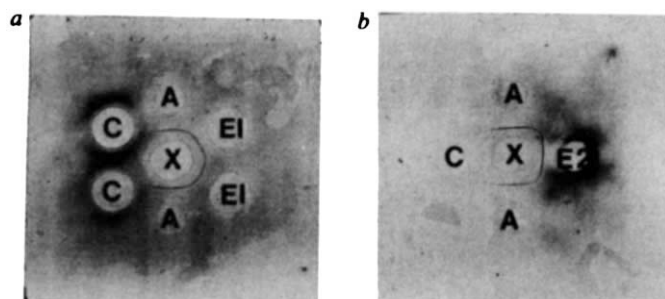


Fig. 1 Immunodiffusion analysis. *a*, X, anti-TSSE antisera; A, crude preparation of TSSE antigen; both antisera and antigen were prepared by M.S.B.³; C, periplasmic preparation from a non-TSSE-producing *E. coli* transformant; E1, periplasmic preparation from RN4506 containing the TSSE-producing clone pRN6100 (see Fig. 2*a*); *b*, E2, supernatant preparation from *S. aureus* RN4512 which contains the TSSE plasmid pRN6201.

Methods: *E. coli* cells (C, E1) were concentrated 100-fold by centrifugation and subjected to osmotic shock to release the periplasmic material¹⁵; *S. aureus* cells were removed by centrifugation. The supernatant was concentrated by precipitation with 4 vol cold ethanol and redissolved in distilled water. The material from both preparations was dialysed against 2 mM Tris buffer, pH 7.5, and the dialysate was concentrated by lyophilization.

conversion. UV- or mitomycin C-induced lysates^{6,7} were diluted and plated on each of two TSSE-negative indicator strains, 8325-4 (=RN450)⁶ and 1030 (a strain sensitive to most *Staphylococcus aureus* phages). Seventeen of the 20 phage preparations gave single plaques on at least one of the two indicators, 16 on strain 1030 and 9 on 8325-4. Three lysates produced no plaques. Lysogens were isolated by streaking growth from the centres of individual plaques, purifying single colonies and scoring for the production of phages able to plate on the original indicator strains. One to three independent lysogens were obtained for each of the 17 lysates and all were immune to the respective donor phage by spot testing.

All the newly isolated lysogens were TSSE negative by immunodiffusion. The two recipient strains were verified as negative; all 20 of the donor strains were verified as TSSE positive, as was strain 1830, used as a recipient strain by Schutzer *et al.*⁵. The possibility that TSSE is actually carried by phage

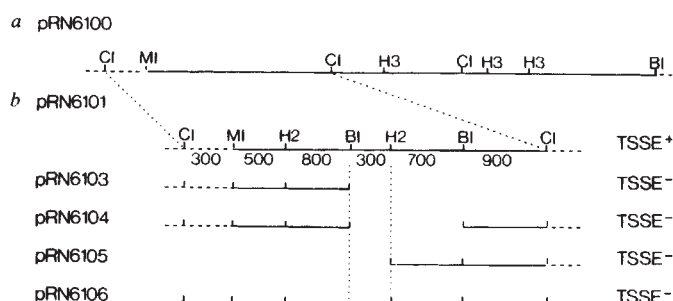


Fig. 2 Mapping the TSSE gene. BI, *Bam*HI; CI, *Cla*I; H2, *Hinc*II; H3, *Hind*III; MI, *Mbo*I. Solid lines represent staphylococcal insert DNA. Horizontal broken lines represent pBR322 DNA. Vertical dotted lines represent a 300-base *Hinc*II-*Bam*HI fragment missing in TSSE-negative subclones. DNA fragment sizes are in base pairs.

Methods: Plasmid DNA was isolated²⁰, restricted and separated by agarose gel electrophoresis. The desired fragment was extracted from the gel²¹, re-ligated and used to transform a suitable recipient strain with selection for pBR322-determined ampicillin resistance. Alternatively, the plasmid DNA was restricted and the mixture of fragments re-ligated and used for transformation. Clones were scored for TSSE production by immunodiffusion and their structures determined by restriction analysis.

but that test strains are unable to express it was eliminated by the introduction of the cloned TSSE determinant (see below) into RN4220, a derivative of RN450. This strain produced ample amounts of TSSE.

The majority of TSS strains express a series of phenotypic traits in common and have similar phage-typing patterns^{4,8}. This indicates that there is a common biotype associated with TSS and our results suggest, in confirmation of the results of Schutzer *et al.*⁵, that this biotype is associated with prophages which have similar plating characteristics. This includes strains such as 2393 which have this biotype but do not produce TSSE. These phages clearly do not carry the TSSE determinant. As *S. aureus* strains are often polylysogenic, it is possible that TSSE is carried by a subsidiary phage in an occasional strain. Indeed, our purpose in presenting these data was not to rule out rigorously the possibility that TSSE is ever carried by a prophage, but rather to show that the existence of phages with similar plating charac-

Table 1 Lysogeny tests with phages from TSSE-producing *S. aureus* strains

Strain	Source	Phage type	Forms plaques on 8325-4	No. lysogens tested	Forms plaques on 1030	No. lysogens tested	No. TSSE+ lysogens
587	CDC-Vag	52	—		—		
033	CDC-Vag	81/29±	—		+	1	0/1
189	CDC-Vag	29/52/81	+	1	+	1	0/2
MN-8	UM-Vag	29	—		+	1	0/1
MN-7	UM-Vag	29	+	1	+	1	0/2
MN-Ba	UM-Vag	29/52/79	—		+	1	0/1
LA-An	UM-Vag	NT	—		+	1	0/1
LA-Ts	UM-Toe	29	+		+	1	0/1
LA-Mo	UM-Vag	29	—		—		
NZ-39	UM-Vag	29/77/80/84	—		+	1	0/1
2-8	UW-Vag	29/52+	+		+	3	0/3
2-11	UW-Vag	29/52+	—		+	1	0/1
4-26	UW-Vag	29/52	+	1	+	1	0/2
4-30	UW-Vag	29/52+	+		+	1	0/1
RN3984	UM-Vag	29/52A/79	—		—		
3-14	UW	29/52A	+	1	+		0/1
RN3955	CDC	29	—		+	3	0/3
RN3985	UM-Vag	NT	+	1	+		0/1
RN3986	UM-Vag	NT	—		+	1	0/1
RN3987	UM-Cerv	29/79	—		—		
2393	PHLS	29/42E	+	1	—		0/1

Strain 2393 is a non-TSSE-producing strain received from Maureen de Saxe. Sources: CDC, Center for Disease Control; UM, University of Minnesota; UW, University of Wisconsin; PHLS, Public Health Laboratory Service, London, UK; Vag, vaginal; Cerv, cervical. Strain 8325-4 = RN450, and is a derivative of NCTC 8325 which has been cured of all known prophages. RN161 was used as a representative 1030 host strain.

teristics is not, *per se*, sufficient grounds for concluding that any common trait is likely to be phage mediated. We suggest, therefore, that there is no experimental or theoretical basis for proposing that the ability to cause TSSE is normally transmitted by lysogenic conversion⁵.

In view of the lack of association of the TSSE gene with known chromosomal, plasmid or phage genes, we elected to clone the TSSE determinant directly. To comply with a requirement of the Recombinant DNA Advisory Committee we constructed an α -haemolysin-negative derivative of the TSSE-positive strain 3-14 (see Table 1). Bulk DNA from strain ISP546 (ref. 9), which is α -haemolysin-negative due to a *Tn551* insertion^{9,10}, was used to transform¹¹ strain 3-14 for erythromycin resistance. *Em*^r (*Tn551*-containing) transformants were confirmed as α -toxin negative¹². A partial *Mbo*I digest of chromosomal DNA from one of these, RN4256, was fractionated on a preparative neutral sucrose gradient and a 7–10-kilobase (kb) fraction was isolated and ligated to *Bam*HI-digested alkaline-phosphatase-treated pBR322 DNA. This preparation was used to transform *Escherichia coli* strain 259 (ref. 13) with selection for the pBR322 Ap^r marker. Transformants were screened for insertional inactivation of the pBR322 Tc^r marker and TSSE-positives were identified among these by a colony radioimmunoassay using rabbit anti-TSSE antiserum¹⁴ that had been pre-absorbed with sonicated *E. coli* and iodinated protein A (kindly provided by T. Meyer). TSSE-positive colonies were verified by immunodiffusion with whole-cell lysates and one of these, RN4506, containing plasmid pRN6100, was analysed further. A comparison of periplasmic shockates¹⁵ with a lysate prepared from the shocked cells indicated that most of the antigenic material was in the periplasm, suggesting that it may be secreted in *E. coli* as it appears to be in *S. aureus*. Immunodiffusion analysis¹⁶ of the periplasmic material (Fig. 1) showed a single line of identity for the cloned material with native TSSE prepared independently by M.S.B.³ and P.M.S.¹⁷, suggesting that all three are the same protein.

The cloned insert in pRN6100 (Fig. 2a) is a 10.6-kb staphylococcal chromosomal fragment containing the structural gene for TSSE. The TSSE determinant was localized to the left-most *Cla*I fragment of the insert by subcloning. Analysis of subclones failing to produce TSSE (Fig. 2b) indicated that a 300-base pair (bp) *Hinc*II–*Bam*HI fragment is required for TSSE expression.

The TSSE determinant was re-introduced into *S. aureus* by cloning to a *S. aureus* plasmid, pE194 (ref. 18). A *Cla*I-linearized preparation of pRN6102, containing the *Cla*I fragment shown in Fig. 2b, was ligated to *Cla*I-digested pE194 DNA and used to transform RN4220 for the pE194 *Em*^r marker. Clones having the required plasmid structure were found to produce the exotoxin. As with native *S. aureus* exotoxin-producers, TSSE was released into the culture medium and could be identified by standard immunodiffusion (Fig. 1b). One producing strain, RN4512, contains the plasmid subclone pRN6201. Note that this experiment has generated a pair of isogenic *S. aureus* strains, RN4220(pE194) and RN4220(pRN6201) (=RN4512), differing only in their ability to produce the TSSE exotoxin—which should lend credence to animal studies.

The TSSE antigen purified from RN4512 was indistinguishable biologically and physically from a sample of toxin purified from the original donor strain, 3-14: both had the same pyrogenicity, endotoxin enhancement, mitogenicity and immunosuppression activity in rabbits (data not shown)¹. The cloned antigen produced a single band with a molecular weight of 22,000 on polyacrylamide gel electrophoresis which comigrated with the control antigen and had the same *pI* value.

Blot-hybridization analysis¹⁹ of chromosomal DNA of several *S. aureus* strains with pRN6100 (the primary TSSE-positive clone) and with several of the subclones as probes suggests that the TSSE determinant is part of a larger segment of DNA that is absent or rearranged in TSSE-negative strains. The blotting patterns thus far obtained are complex and have not been fully resolved; a typical example is shown in Fig. 3. Note that the two TSSE-positive strains each have two homologous fragments

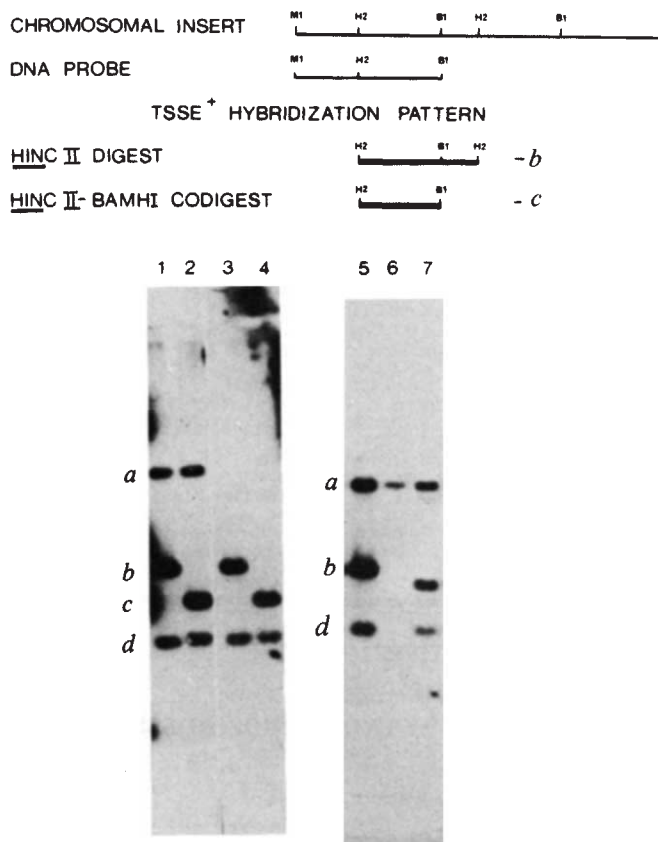


Fig. 3 Genotypic variation between TSSE-producing and -non-producing strains of *S. aureus*. Radioactive DNA probe (pRN6103, Fig. 2b) contains a 1.3-kb *Mbo*I–*Hinc*II chromosomal *S. aureus* fragment which defines the left end of the original TSSE insert. b, c, *Hinc*II and *Hinc*II–*Bam*HI digests respectively of chromosomal DNA from TSSE-producing strains. Hybridization pattern for the following chromosomal digests are shown: lanes 1 and 5, *Hinc*II-digested DNA from RN4256 (donor cloning strain); 2, the same, co-digested with *Hinc*II + *Bam*HI; 3, 4, chromosomal DNA from RN3984, a second TSSE-producing strain digested with *Hinc*II and *Hinc*II + *Bam*HI respectively; 6, 7, *Hinc*II-digested DNA from two non-producing *S. aureus* strains, RN450 and COL²², respectively.

Methods: Chromosomal DNA was prepared²³, restricted and electrophoresed on agarose gels. Restriction fragments were transferred to nitrocellulose and hybridized to ³²P-labelled plasmid DNA by the method of Southern¹⁹. The probe was ³²P-labelled by nick-translation²⁴. The hybridization was performed at 65 °C in the presence of 10% dextran sulphate. The filter was then washed extensively, dried and exposed to X-ray film at –70 °C for 24 h with an intensifying screen.

(b, c) in common, including one that is known to contain part of the TSSE gene (b). Both negative strains lack this fragment and RN450 lacks the d fragment as well. On the other hand, one of the TSSE-positive strains and both negative strains have a larger fragment (a) that shows homology with the probe. The origin and significance of this homology are unclear.

The blotting results collected so far have clearly defined the right end of the TSSE-associated DNA (not shown), but not the other end; a probe containing only the left-most *Mbo*I–*Hinc*II fragment of the pRN6101 insert shows no homology with RN450 DNA (not shown). The results do show, however, that the observed interstrain variability in TSSE production is genotypic; experiments in progress should reveal the extent and structure of the variable genetic segment involved.

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Adrenergic activation of triiodothyronine production in brown adipose tissue

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There are several mechanisms by which homeothermic animals increase heat production, including shivering, sympathetic nervous system activation and stimulation of thyroid hormone secretion. Studies in rats have shown that increased sympathetic activity causes increased heat production in brown adipose tissue (BAT) after cold exposure or food ingestion^{1–3}. Acute cold exposure also increases circulating thyroid hormones⁴ which in turn stimulate cellular metabolism through induction⁵ of various enzymes. Most metabolic effects of thyroxine (T₄) are thought to be due to the triiodothyronine (T₃) which is produced from T₄ by a process of 5' monodeiodination. There are two enzymes responsible for this reaction^{6–8}: type I, or propylthiouracil (PTU)-sensitive iodothyronine deiodinase (5'D-I), which is reduced in hypothyroidism, stimulated in hyperthyroidism and probably provides most of the circulating T₃ in the adult rat⁹. Type II 5'-deiodinase (5'D-II) is characteristic of brain and pituitary, is increased by thyroidectomy, is not inhibited by PTU and provides 50–80% of the intracellular T₃ in these two tissues. Recently, 5'D-II activity was identified in interscapular BAT¹⁰. As the sympathetic nervous system influences the metabolic activation of BAT, we have studied the effects of noradrenaline and acute cold exposure on BAT 5'D-II. We report here that both noradrenaline and cold exposure increase BAT 5'D-II through α_1 -adrenergic receptors, whereas depletion of catecholamines with α -methyl-p-tyrosine (MPT) prevents the effect of cold but not that of noradrenaline. These results suggest that the sympathetic nervous system may increase T₃ production in rats by stimulating BAT 5'D-II. By increasing metabolic rate, this rise in T₃ would enhance the thermogenic response to sympathetic stimulation.

Male Sprague-Dawley rats (75–150 g) were obtained from Charles River Laboratories and maintained on *ad libitum* diets at 23 °C. At various times after injection of catecholamine agonists or antagonists, or after exposure to 5 °C for 4 h, rats were killed by decapitation. BAT was homogenized and assayed

Table 1 Effect of noradrenaline (a) and acute cold stress (b) on type II iodothyronine 5' deiodinase activity in BAT and cerebral cortex

	n	BAT	Cerebral cortex
a 1. Control	5	33 ± 7	38 ± 6.5
NA	5	290 ± 47 (<0.001)	47 ± 9.7 (NS)
2. Control	5	6.2 ± 1.5	—
NA	5	180 ± 44 (<0.01)	—
3. Control	5	12 ± 1	—
NA	5	86 ± 4 (<0.001)	—
b 1. Control	4	17 ± 4	18 ± 2.1
Cold stress	4	82 ± 26 (<0.05)	37 ± 3.5 (<0.005)
2. Control	5	6.2 ± 1.5	—
Cold stress	5	290 ± 90 (<0.025)	—
3. Control	5	13 ± 1.3	29 ± 5.2
Cold stress	5	130 ± 40 (<0.025)	25 ± 4.3 (NS)

a, Treatment with noradrenaline (NA; 40 µg per 100 g body weight) given s.c. at –2 h. b, Treatment at 5 °C for 4 h. Values shown are fmol I[–] released per h per mg protein ± s.e.m. NS, not significant.

for 5'D-II as described previously¹⁰. Activity in all samples from a given experiment was measured simultaneously using 2 nM [5'-¹²⁵I]reverse T₃ (rT₃) as substrate in the presence of 1 mM PTU and 10 mM dithiothreitol. In some experiments, T₄ to T₃ conversion was also assayed by measuring the ¹²⁵I-T₃ produced from ¹²⁵I-T₄ (ref. 7). Protein content of the BAT infranant was quantitated by Lowry's method. Catecholamine agonists and antagonists were obtained from commercial sources.

Noradrenaline caused a 10-fold (range 6–30) increase in the BAT 5'D-II activity 2 h after subcutaneous (s.c.) injection (Table 1). The increased activity reached a plateau between 4 and 8 h after noradrenaline injection, but had returned to normal by 16 h. Noradrenaline did not stimulate 5'D-II in cerebrocortical or pituitary tissue. There was also a significant increase in 5'D-II activity during 4 h at 5 °C. Cerebrocortical 5'D-II was not consistently affected by cold stress. The effects of cold and noradrenaline on T₄ to T₃ conversion in BAT were identical to those on PTU-insensitive rT₃ 5' deiodination. Serum T₄ and T₃ concentrations were increased during cold exposure and not consistently altered after noradrenaline treatment.

The experiments shown in Table 2 examined the effects of various adrenergic antagonists on BAT 5'D-II responses. Phentolamine, but not propranolol, partially blocked the increased 5'D-II induced by noradrenaline. Phentolamine given to hypothyroid rats according to the schedule in Table 2, had no effect on the elevated 5'D-II activity typical of this state, arguing against a nonspecific effect of this agent on the assay. The α_1 -antagonist prazosin significantly attenuated the response of BAT to noradrenaline, but yohimbine, an α_2 -adrenergic blocking agent, had no effect. The effect of cold on 5'D-II in BAT was also inhibited by prazosin, but not by yohimbine. This inhibition could not be explained by an *in vitro* effect, as the assay was not affected by 100 µM prazosin added *in vitro*, and the increase in 5'D-II induced by 4 h of cold exposure was not inhibited by prazosin at 0.4 mg per 100 g body weight injected intravenously (i.v.) at the end of this period. Pretreatment with MPT eliminated the cold-induced increase in BAT 5'D-II, but had no effect on the response to noradrenaline.

These results show that noradrenaline or cold stress causes a rapid increase in 5'D-II in BAT homogenates, presumably due to activation of latent enzyme. BAT 5'D-II is increased when serum T₄ and T₃ levels fall after thyroidectomy¹⁰ or hypophysectomy (our unpublished observations). However, as noradrenaline did not consistently alter serum thyroid hormone levels, and cold stress increased serum thyrotrophic hormone (TSH), T₄ and T₃, the observed increase in 5'D-II after these experimental perturbations is probably not mediated by the pituitary-thyroid axis.

The 5'D-II increase after noradrenaline injection was inhibited selectively by α_1 -adrenergic receptor antagonists while that after cold stress was prevented by catecholamine depletion

Table 2 Effects of catecholamine antagonists on the response of type II iodothyronine 5' deiodinase of BAT to noradrenaline and cold stress

Expt	Antagonist dose per 100 g body weight	5'D-II stimuli	
		40 µg NA per 100 g at time 0, BAT obtained at 2 h	BAT obtained after 4 h at 5 °C
Expt a	Phentolamine	34 ± 5 (<0.01)	39 ± 9 (NS)
	Propranolol	121 ± 18 (NS)	—
Expt b	Prazosin	44 ± 5 (<0.001)	2.6 ± 0.3 (<0.001)*
	Yohimbine	83 ± 7 (NS)	100 ± 9 (NS)
Expt c	MPT	81 ± 17 (NS)	12 ± 4 (<0.025)

Results are expressed as per cent (±s.e.m.) of the stimulated control; comparisons were by unpaired Student's *t*-tests; *n* = 5 for each group. i.p., Intraperitoneal. NS, not significant.

* Prazosin (0.4 mg per 100 g i.v.) 10 min before cold exposure; 0.2 mg per 100 g i.p. after 2 h in cold. All controls were given vehicle at similar times.

or α_1 blockade. Although we do not know whether the 5'D-II activity is present in the fat cells or in some other component of the tissue (for example, nerve terminals or capillary endothelium), there is previous evidence for α_1 -adrenergic receptors in BAT¹¹. It is also conceivable, although we believe unlikely, that the 5'D-II response is the result of an α_1 effect elsewhere in the animal. The lack of response of the 5'D-II in pituitary and cortex to these manipulations suggests tissue specificity in this response.

An increase in BAT 5'D-II could contribute to the higher serum T₃-T₄ ratios observed in cold-adapted and hyperphagic rats¹²⁻¹⁴. It could also explain the abrupt increase in T₃ observed after refeeding fasted rats¹⁴, an effect which can be mimicked by noradrenaline injection. As BAT is present in greater quantities in young than mature animals, BAT 5'D-II could also account for our recent observation that almost all of the T₄ to T₃ conversion in 2-week-old rats occurs by a PTU-insensitive process (J.E.S., in preparation). In mature euthyroid rats, only 30-40% of the extrathyroidal T₄ to T₃ conversion is PTU-insensitive^{15,16} and this fraction would be the upper limit of the contribution of BAT 5'D-II to extrathyroidal T₃ production in adult rats.

While a physiological role for BAT in adult man is still a matter for debate (see refs 1-3, 17), catecholamine activation of 5'D-II in the newborn could explain the abrupt, marked increase in serum T₃ occurring after birth¹⁸. In the newborn sheep, a similar T₃ increase can be dissociated from the post-delivery TSH spurt. This increase in serum T₃ in the sheep is virtually eliminated by MPT pretreatment¹⁹, analogous to the effect of this agent on the cold-induced increase in BAT 5'D-II in the present study. Given the significant quantity of BAT in the human newborn, such an explanation deserves as much consideration as an acute postnatal increase in 5'D-I in liver and kidney¹⁹. Lastly, short-term overfeeding of human adults increases peripheral T₄ to T₃ conversion by 76% (ref. 20). This increase could also be attributed, in part, to sympathetic activation of BAT 5'D-II in association with diet-induced thermogenesis. Taken together, these results indicate that there is a synergistic relationship between thyroid hormones and the sympathetic nervous system in the rat and, perhaps, in man, which could amplify the thermogenic response.

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Enzyme replacement in grafted nerve of twitcher mouse

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'Twitcher' is a recently recognized mouse mutant which, on the basis of morphological¹ and enzymatic² criteria, represents a murine model for globoid-cell leukodystrophy in man³. In the twitcher mouse, myelin sheaths develop normally in peripheral nerves until about the fifteenth day when the rate of myelination declines, demyelination begins and Krabbe-type inclusions are seen in macrophages and Schwann cells⁴. With age, demyelination becomes extensive, affecting fibres of all sizes. The axons are relatively spared, although they are smaller than normal. Twitcher peripheral nerves transplanted into normal hosts show, after 2 months, all the characteristics associated with globoid-cell leukodystrophy⁵. After longer periods of time, however, pathological changes within the grafts appear considerably improved with only occasional evidence of myelin loss, little endoneurial oedema and few globoid cells⁵. Migration of host Schwann cells into the graft can be excluded as a possible explanation⁶. In the experiments reported here, we have attempted to determine whether the morphological improvement of grafted twitcher nerves is accompanied by an increase in activity of the enzyme galactosylceramidase. Our results show that galactosylceramidase activity in twitcher sciatic nerves grafted into normal hosts is variable after 1-2 months but indistinguishable from those in the host nerves and much higher than those in intact twitcher nerves after 4.5-9 months. In addition to migration of host Schwann cells, other tissues originating from the host can be excluded as cause of the high enzyme activity. This is the first evidence of long-term *in vivo* enzyme replacement, accompanied by improved pathology, in a genetic sphingolipidosis.

Table 1 Galactosylceramidase activity in twitcher graft and host sciatic nerves

Time	Experiment	Specimen (n)	Galactosylceramidase (nmol per h per mg protein $\pm$ s.d.)
1-2 months	N-twi-N	Graft (4)	0.43 $\pm$ 0.43
		Contralateral (4)	2.04 $\pm$ 0.55
	N-N-N	Graft (2)	1.01, 0.70
		Contralateral (2)	1.14, 1.78
4.5-9 months	N-twi-N	Proximal (4)	2.17 $\pm$ 1.09
		Graft (4)	2.74 $\pm$ 1.28
		Distal (4)	2.08 $\pm$ 0.59
		Contralateral (3)	1.31 $\pm$ 0.09
	N-N-N	Proximal (2)	2.17, 1.08
		Graft (2)	3.01, 2.80
		Distal (2)	2.49, 3.48
		Contralateral (2)	1.00, 2.01
	Twitcher, untreated (5)		0.17 $\pm$ 0.06

N-twi-N, twitcher nerve grafted into normal host; N-N-N, normal nerve grafted into normal host. The average $\pm$ s.d. for the 12 N-N-N specimens combined is 1.89 ± 0.88 . For technical reasons biochemical examination of proximal and distal segments at 1 and 2 months was not carried out.

For this study (CE/J $\times$ C57BL/6J) F_1 mice were used as hosts to avoid rejection of grafts from twitcher mice which had been produced in our laboratory and originated from the same type of mating. Nerve grafts were carried out using a technique described previously^{5,6}. As controls, sciatic nerves of normal mice were grafted into normal hosts. After 1-9 months, recipient mice were anaesthetized with pentobarbitone and the graft and segments of proximal and distal stumps as well as the contralateral sciatic nerve carefully removed. The grafts were separated from the proximal and distal stumps and the newly formed collagen and the epineurium removed using a dissecting microscope. Care was taken not to include the border area between the graft and the host nerves in any one of the segments. Fragments of sciatic nerve were also removed from intact twitcher mice. Each segment was frozen separately and sent from London to New York. Mice receiving twitcher and normal nerve grafts were perfused with Karnovsky's fluid after 4 and 5 months and used as morphological controls.

The frozen specimens were number-coded and biochemical studies were carried out blindly without knowledge of the nature of the specimen. The specimens weighing approximately 1-3 mg wet weight were homogenized in a motor-driven all-glass homogenizer in 0.5 ml of water. Aliquots (0.1 ml) were used for galactosylceramidase assay according to the standard procedure² except that the incubation time was 6 h. This long incubation time was a compromise regimen that allowed for the limited size of the specimens and ensured that reliable amounts of ^3H -galactose were released by enzymatic reaction. Preliminary experiments indicated that the 6-h incubation resulted in calculated activities that were 20-30% lower than those determined within the strictly linear range of the reaction. Remaining homogenates were used for protein determination⁷ and for assays of control enzymes, 4-methyl-umbelliferyl β -galactosidase and 4-methylumbelliferyl β -N-acetylglucosaminidase⁸.

Light and electron microscopic examination of the twitcher nerve grafts confirmed previous findings⁵ and suggested that at 4 and 5 months grafted twitcher Schwann cells produce apparently normal myelin.

The results of galactosylceramidase activities measured at 1 and 2 months (Table 1) were rather contradictory. At both times one twitcher graft showed no enzyme activity while another showed a level of enzyme comparable with that of normal grafts. Statistical analysis of enzyme levels at these stages showed a slightly higher activity in twitcher grafts than in untreated nerves, but the difference was not significant ($0.1 > P > 0.05$). By contrast, at 4.5-9 months, enzyme activity in twitcher grafts was

significantly higher than in untreated nerves ($P < 0.01$) and not significantly different from the level of enzyme in normal grafts ($P > 0.01$). In normal nerves the activities of the control enzymes were similar in the proximal, middle and distal segments.

The results of the experiments show that, at an early stage, twitcher nerves grafted into normal hosts present variable levels of enzyme activity that might be explained by the time the enzyme takes to accumulate in the graft and to become effective. The extremely low levels or absence of enzyme at these early stages are in keeping with the expression of characteristic twitcher morphology seen in graft experiments at 2 months⁵. With the longer survival times twitcher nerves grafted into normal hosts showed a persistently normal activity of galactosylceramidase. However, the assays were done on the whole graft and it is therefore possible that the enzyme is localized in tissue constituents other than twitcher Schwann cells, such as Schwann cells migrated from the host nerve, abundant scar tissue around the graft, increased numbers of endoneurial macrophages of host origin, or axonal elements which are naturally of host origin. Our previous observations^{5,6} as well as the grafts from the present series used for morphology seem to rule out these possibilities: host Schwann cell migration was not observed in either normal-twitcher-normal or Trembler-twitcher-Trembler grafts. Epineurial scar tissue is minimal or absent if graft rejection is avoided by using twitcher littermates as hosts⁵ or immunosuppressants⁶. After 4-6 months there are only occasional macrophages present within the graft. Finally, axonal elements are not expected to contain any significant lysosomal hydrolase activity. Thus the present results seem to provide good biochemical support for the suggestion that, by whatever mechanism, normal galactosylceramidase is taken up by twitcher Schwann cells, enabling them to function apparently normally. Although the bulk of tissue galactosylceramidase is tightly bound to lysosomes it is known to be present in serum^{9,10}. Even a small amount of enzyme in the extracellular fluid could be taken up and reach a level adequate for normal functioning of the cell.

Several attempts are being made to correct genetic lysosomal enzyme deficiency by enzyme replacement (see refs 11-13 for recent review). Cultured cells such as fibroblasts are known to take up exogenously added enzymes that are absent due to genetic defect. Enzyme replacement *in vivo* by various techniques has been successful in reducing the level of stored materials or increasing the level of the enzyme. In some instances clinical improvement has been reported. Use of partial exchange transfusion in a patient with genetic adenosine deaminase deficiency resulted in temporary clinical improvement¹⁴. More recently Hobb *et al.*¹⁵ reported clinical improvement following bone-marrow transplantation in a patient with Hurler disease. An apparent long-lasting increase of β -glucuronidase activity was reported in liver and serum of β -glucuronidase-deficient mice after bone-marrow transplant but morphological findings were not included¹⁶.

Our present results and previous morphological data^{5,6} suggest that twitcher Schwann cells in the graft can take up normal galactosylceramidase from the host tissue and become capable of forming and maintaining myelin sheath normally. While the present experimental design is not directly applicable to human patients, this authentic model of a human sphingolipidosis is amenable to many other experimental manipulations to explore possibilities for enzyme replacement therapy.

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Neuropeptides modulate the β -adrenergic response of purified astrocytes *in vitro*

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Neuropeptides may have functions in the central nervous system (CNS) other than altering neuronal excitability. For example, they may act as regulators of brain metabolism by affecting glycogenolysis¹. Since it has been suggested that glial cells might provide metabolic support for neuronal activity^{2,3}, they may well be one of the targets for neuropeptide regulation of metabolism. Consistent with this view are reports that peptide-containing nerve terminals have been seen apposed to astrocytes^{4,5}, but it is also quite possible that peptides could act at sites lacking morphological specialization^{6,7}. Primary cultures containing CNS glial cells have been shown to respond to β -adrenergic agonists with an increase in cyclic AMP and, as a result, with an increase in glycogenolysis^{8,9} and have also been shown to respond to a variety of peptides with changes in cyclic AMP^{10–12}. In the study reported here, we have examined the effects of several peptides on relatively pure cultures of rat astrocytes. We demonstrate that the increase in intracellular cyclic AMP induced by noradrenaline is markedly enhanced by somatostatin and substance P and is inhibited by enkephalin, even though these peptides on their own have little or no effect on the basal levels of cyclic AMP. Vasoactive intestinal peptide (VIP) on the other hand increases cyclic AMP in the absence of noradrenaline. These results suggest that neuropeptides influence glial cells as well as neurones in the CNS and, in the case of somatostatin and substance P, provide further examples of neuropeptides modulating the response to another chemical signal without having a detectable action on their own.

Astrocyte cultures used in these experiments were prepared by a modification of the method of McCarthy and de Vellis¹³. Briefly, the cerebral cortex of newborn W/Fu rats was dissected free of meninges, dissociated into single cells with trypsin and collagenase, plated onto polylysine-coated tissue culture flasks and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine and 25 μ g ml⁻¹ gentamycin as previously described¹⁴; cultures were fed every 3 days. After 10 days, cultures were placed on an orbital shaker overnight to remove cells growing on top of the astrocyte monolayer (neurones, oligodendrocytes, fibrous astrocytes and macrophages). The remaining adherent cells were treated with cytosine arabinoside (10^{-5} M for 48 h) to kill rapidly dividing cells and then removed from the flasks with trypsin and EDTA, and replated either into 16-mm multiwell plates (Linbro) at a density of 20,000 cells per well, or onto polylysine-coated glass coverslips. The cells were then grown for a further 3–5 days without cytosine arabinoside before being used in the cyclic

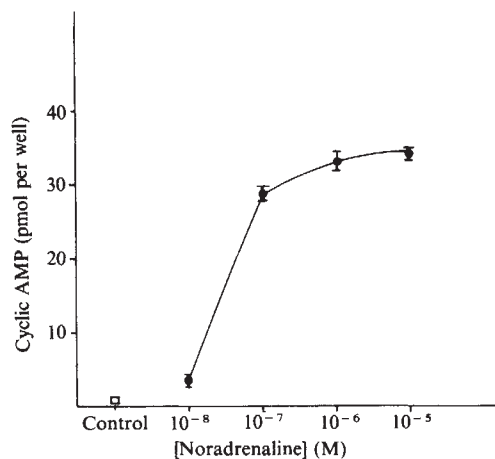


Fig. 1 Intracellular cyclic AMP after a 5 min exposure to various doses of noradrenaline (mean $\pm$ s.e.m., $n=4$). In this and the following experiments, multiwell plates (into which 20,000 astrocytes per well had been plated 3–5 days before the experiment) were washed free of growth medium and equilibrated in a balanced salt solution (BSS) for 30 min before the experiment in a warm room maintained at 37 °C. [The BSS contained 131 mM NaCl, 3 mM KCl, 3.7 mM CaCl₂, 3.6 mM Mg(SO₄)₂, plus 25 mM HEPES adjusted to pH 7.4 with 12.5 mM NaOH and 0.1% bovine serum albumin (RIA grade, Sigma). Total osmolality = 285 mosmol kg⁻¹]. This solution was removed and 0.3 ml of BSS containing various drugs was added to each well. At various times the drug solution was removed and 0.3 ml of 2 M HClO₄ was added immediately to extract the cyclic AMP (all manipulations were done at 37 °C). The plates were then stored at -20 °C prior to assay. Aliquots of the HClO₄ extract were succinylated and then assayed for cyclic AMP¹⁸. Each point on the graphs represents the mean $\pm$ s.e.m. of either quadruplicate or triplicate wells as indicated by n in each figure. Cyclic AMP assays of each well were done in duplicate and repeated if values did not agree to within 10%.

AMP experiments (multiwells) or tested for purity using indirect immunofluorescence (coverslips). Rabbit antisera to glial fibrillary acidic protein (GFAP) and fibronectin (FN) were used to identify astrocytes and fibroblasts (or leptomeningeal cells), respectively^{14,15}, while monoclonal antibodies to neurofilament protein (NF)¹⁶ and to galactocerebroside (Gal-C)¹⁷ were used to identify neurones and oligodendrocytes, respectively; normal rabbit serum (NRS) was used to label macrophages¹⁴. The cultures used in the present experiments contained >95% GFAP+ cells, no NF+ cells, 0–3% Gal-C+ cells, <1% NRS+ cells and <5% FN+ cells.

Cyclic AMP was measured in 2 M HClO₄ extracts of the astrocyte cultures by radioimmunoassay according to the method of Cailla *et al.*¹⁸. The basal level of cyclic AMP in nine different experiments was 1.13 ± 0.48 (mean $\pm$ s.d., $n=35$) pmol per 20,000 astrocytes plated. Figure 1 shows the increase in intracellular cyclic AMP with increasing doses of noradrenaline after a 5-min exposure (half maximum dose $\sim 5 \times 10^{-8}$ M noradrenaline). The increase in cyclic AMP induced by saturating concentrations of noradrenaline (10^{-6} M or 10^{-5} M) after 5 min was in the range 20–109-fold over nine different experiments; the variability in this increase from experiment to experiment reflects the fact that we were looking at the initial rate of cyclic AMP accumulation (see Fig. 3).

As shown in Fig. 2, addition of somatostatin by itself had little effect on cyclic AMP levels; (in some experiments, there was a small decrease, <0.5 pmol after addition of 10^{-7} – 10^{-6} M somatostatin, but the decrease was not dose dependent). However, when somatostatin was added together with 10^{-5} M noradrenaline, the increase in cyclic AMP was much greater (60-fold in 5 min with 10^{-7} M somatostatin) than when 10^{-5} M noradrenaline was added on its own (20-fold in 5 min). The potentiation of the noradrenaline response by somatostatin was

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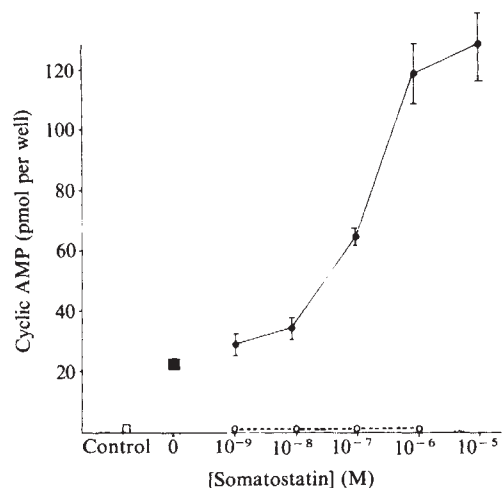


Fig. 2 Dose-response curve for somatostatin with (●) and without (○) the addition of 10^{-5} M noradrenaline. The open square indicates the control and the closed square indicates the effect of 10^{-5} M noradrenaline without the addition of somatostatin. Each point represents the amount of intracellular cyclic AMP in the astrocytes after a 5-min exposure (mean $\pm$ s.e.m., $n = 4$). Cyclic somatostatin was obtained from Sigma.

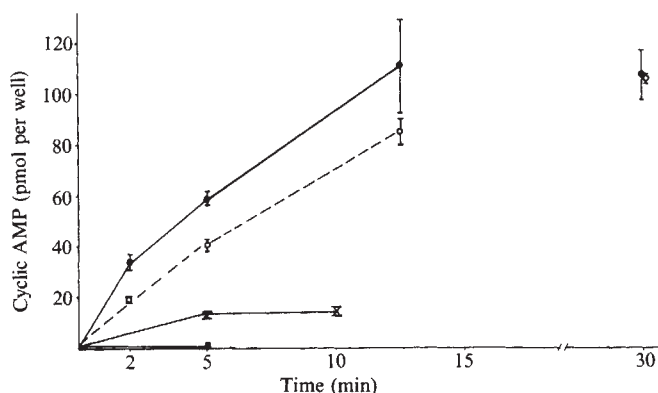


Fig. 3 Time course of the increase in intracellular cyclic AMP in astrocytes after exposure to either 10^{-5} M noradrenaline (○), 10^{-5} M noradrenaline plus 10^{-7} M somatostatin (●), 10^{-7} M somatostatin alone (■) or to 10^{-7} M VIP alone (×). Each point is the mean $\pm$ s.e.m., $n = 3$ or 4.

dose-dependent with a half-maximum effect at approximately 10^{-7} M somatostatin. Significant potentiation of the noradrenaline response by somatostatin was seen in 7 out of 8 experiments (Student's *t*-test, $P < 0.05$).

We examined the kinetics of cyclic AMP accumulation in response to noradrenaline alone and together with somatostatin. As shown in Fig. 3, the increase in cyclic AMP in response to noradrenaline was linear for the first 5 min and started to plateau after 12.5 min. When noradrenaline was applied in the presence of somatostatin, the rate of cyclic AMP accumulation was increased although the amplitude of the response at 30 min was not changed. Thus somatostatin seems to affect the kinetics of the response to noradrenaline and this potentiating effect is more apparent at early time points.

In the presence of the β -adrenergic antagonist ($\pm$)propranolol (5×10^{-5} M), the response to noradrenaline (10^{-5} M) and to noradrenaline (10^{-5} M) + somatostatin (10^{-7} M) was completely blocked indicating that the increase in cyclic AMP was mediated by a β -adrenergic receptor; moreover, in the presence of propranolol there was no indication of the presence of inhibitory α receptors. In addition, although intracellular cyclic AMP

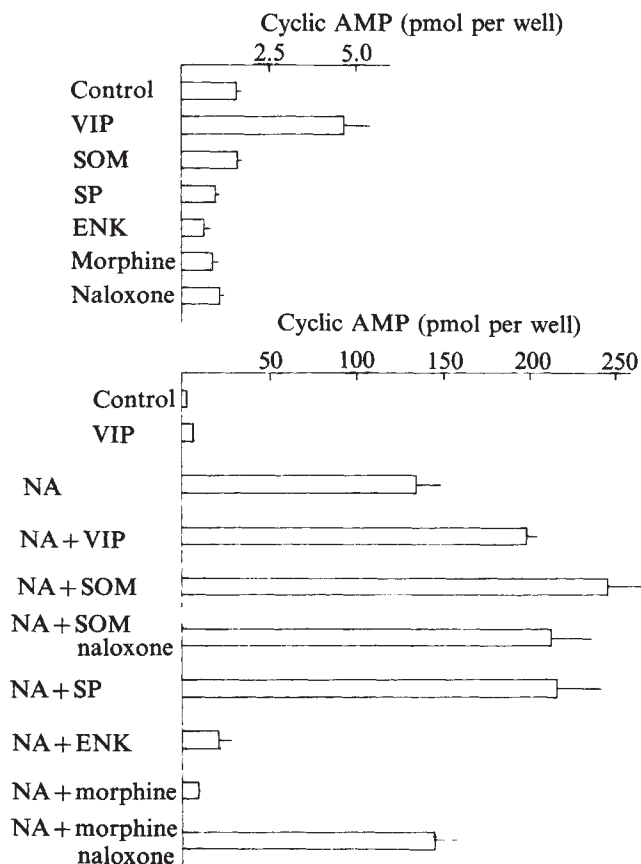


Fig. 4 Intracellular cyclic AMP in astrocytes after a 5-min exposure to various drugs: 10^{-7} M somatostatin (SOM), 10^{-7} M substance P (SP), 10^{-7} M VIP, 10^{-7} M [Met]enkephalin (ENK), 10^{-5} M morphine, 10^{-5} M naloxone. (All peptides were synthetic, from Sigma). The top panel shows the effect of the drugs in the absence of noradrenaline. The bottom panel shows the effect of the drugs in the presence of 10^{-5} M noradrenaline (NA) (mean $\pm$ s.e.m., $n = 4$). Data were analysed by one-way analysis of variance and the Student range test. When compared to cyclic AMP basal level, VIP alone induced a significant increase ($P = 0.05$) in cyclic AMP. When compared to NA alone, NA + VIP, NA + SP, NA + SOM and NA + SOM + naloxone resulted in significantly higher levels of cyclic AMP ($P \leq 0.05$). In contrast, cyclic AMP levels in the presence of NA + met-ENK or NA + morphine were significantly lower than in the presence of NA alone ($P \leq 0.005$). Cyclic AMP levels in the presence of NA + naloxone or NA + morphine + naloxone were not significantly different to NA alone.

levels were increased by addition of prostaglandin E_1 , somatostatin at 10^{-6} M did not potentiate this response (although in sister cultures 10^{-7} M somatostatin did potentiate the noradrenaline response). Thus the potentiating effect of somatostatin is not seen with all agonists that activate adenylate cyclase.

We also examined the effect noradrenaline and somatostatin on cultures of leptomeningeal cells and fibroblasts (from skin), as these were thought to be the major contaminating cell types in our astrocyte cultures. These cultures were obtained by dissociating either meninges or dorsal skin from new born rats¹⁴, growing the cells to confluence and then replating them in a similar fashion to the cortical astrocytes. The leptomeningeal cells were $>95\%$ Ran-2+ (17), $<2\%$ GFAP+ and the skin fibroblasts were 100% GFAP-; both types of cells were intensely FN+. The basal level of cyclic AMP was lower in these cells than in the astrocyte cultures (~ 0.1 pmol per 20,000 cells plated) and the response of fibroblasts to noradrenaline (or to somatostatin + noradrenaline) was relatively small (~ 2 -fold in 5 min) and leptomeningeal cells did not respond at all. Thus it is unlikely that these contaminating cells contributed sig-

nificantly to the astrocyte responses shown in Figs 1–3. It is important to note that our cultures contained very few macrophages and no neurones, since both of these cell types are potential targets for noradrenaline and peptide effects on cyclic AMP.

The effects of substance P, VIP, [Met]enkephalin and morphine are shown in Fig. 4. The response to substance P was similar to that of somatostatin, having little effect by itself but potentiating the increase in cyclic AMP induced by noradrenaline. On the other hand, 10^{-7} M VIP alone increased the basal level of cyclic AMP about 4-fold with a maximum response seen within 5 min (Fig. 3); however, when VIP and noradrenaline were applied together, there was a synergistic effect and the resulting increase in cyclic AMP was greater than the sum of the responses to VIP and noradrenaline alone. In contrast, both [Met]enkephalin and morphine produced a small decrease in basal cyclic AMP when applied alone, and when applied in the presence of noradrenaline, they dramatically inhibited the noradrenaline-induced increase in cyclic AMP. This effect of morphine was antagonized by the opiate antagonist, naloxone.

One likely consequence of noradrenaline-induced increases in cyclic AMP in CNS tissue is an increase in glycogenolysis. Both noradrenaline and, more recently, VIP have been shown to stimulate glycogenolysis in brain slices^{1,19}; however, the target cell types involved have not been identified. Astrocytes are thought to store much of the brain's glycogen^{2,3} and glial cell lines^{8,20} respond to β -adrenergic agonists with increased glycogen breakdown and inhibition of glucose uptake. Therefore, it is possible that the cells predominantly involved in glycogenolytic responses (as well as in cyclic AMP changes) are astrocytes rather than neurones. Consistent with this view are the very large changes in cyclic AMP seen in these cultured astrocytes compared to fibroblasts or leptomeningeal cells which parallel the large cyclic AMP changes typical of CNS tissue compared with other tissues²¹. The present experiments suggest that not only noradrenaline and VIP, but a number of other peptides—somatostatin, substance P and enkephalin—may be involved in regulating brain metabolism by a direct action on astrocytes.

Van Calker and his colleagues^{10–12} have reported that peptides can regulate cyclic AMP accumulation in cultured rat and mouse brain cells and have also suggested that glial cells may be targets for peptide action. However, their results differ from ours in several respects: while they also find that VIP stimulates cyclic AMP accumulation in cultured brain cells, they report that somatostatin inhibits cyclic AMP accumulation and that opiates and substance P have no effect. Although the reasons for this discrepancy are uncertain, differences in the culture conditions (for example their cultures were fed infrequently and contained a relatively high proportion of macrophages¹⁰) and the fact that their experiments were carried out in the presence of isobutyl methyl xanthine (IBMX) are probably important. In addition, the responses described in our paper were more pronounced at short time points (<5 min) and were not apparent at 30 min.

We are interested in the mechanisms involved in the neuropeptide effects described above. In the case of VIP and [Met]enkephalin, it is possible that there is direct coupling of the peptide receptors to either the stimulatory or inhibitory forms of the nucleotide-binding, regulatory subunit (G protein) of adenylate cyclase²². However, in the case of somatostatin or substance P, this type of mechanism seems unlikely since one would then expect to see a substantial increase in cyclic AMP when either somatostatin or substance P was applied alone, given their large potentiation effects. It is possible that somatostatin and substance P could either (1) affect the binding of noradrenaline to the β -adrenergic receptor or (2) affect the coupling of the β -receptor to the G protein. There are other examples where peptides apparently modulate the response of another chemical ligand without having measurable effects on their own: in adrenal medullary cells, substance P and somatostatin inhibit nicotinic responses^{23,24}, and in the salivary gland

VIP potentiates muscarinic responses²⁵. In addition, benzodiazepines potentiate GABA-induced responses without having an effect by themselves²⁶. In each of these examples there is some evidence that there may be interaction between receptors^{27–29}. We are now doing radio-binding studies to look for possible interactions between somatostatin or substance P receptors, the β -adrenergic receptor and the G protein.

The fact that all four peptides we tested had effects on cultured astrocytes suggests that these cells have receptors for the majority of neuropeptides. It is possible that this is also true for astrocytes *in situ* and that their function is regulated by the particular peptide(s) released in their vicinity at a particular time. It may be difficult to demonstrate peptide receptors on astrocytes *in situ*, especially if they are present in low numbers and/or diffusely distributed on the cell surface. Nevertheless, our results support the idea¹⁰ that astrocytes as well as neurones should be considered when assessing the functions of neuropeptides in the CNS.

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Asymmetric relationships between homosynaptic long-term potentiation and heterosynaptic long-term depression

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All synaptically-based neuropsychological theories of learning postulate that there are changes resulting from neural activity which are long-lasting and confined to specific sets of synapses^{1–3}. In the past decade a form of synaptic strengthening known as long-term potentiation (LTP) has been found which results from high-frequency neural activity and is of sufficient duration to model as a learning mechanism^{4,5}. Some early tests of the synaptic specificity of LTP in area CA1 of the hippocampus indicated that although LTP was specific to the tetanized pathway, in a converging untetanized pathway it was associated with depression of synaptic transmission lasting for at least

30 min⁶⁻⁸. However, others have found that this heterosynaptic depression more usually decays within 5–15 min post-tetanus despite the maintenance of LTP in the tetanized pathway⁹⁻¹³. Similarly, in the dentate gyrus (DG), LTP of either the lateral (LPP) or medial (MPP) components of the perforant path afferents has been associated with only short-lasting reciprocal heterosynaptic depression¹⁴. Here, using more detailed measurement of stimulus intensity curves, we report that tetanization of either MPP or LPP reliably depresses synaptic transmission in the other pathway for at least 3 h. This heterosynaptic depression, considerably smaller than the usual magnitude of LTP, was obtained regardless of whether LTP had been produced in the tetanized homosynaptic pathway. Heterosynaptic long-term depression was not observed if the test pathway had been previously tetanized.

In each of 16 rats under barbiturate anaesthesia, a 75- μ m stainless-steel recording electrode was lowered stereotactically into the hilus of each hemisphere. In the experimental hemisphere, two 125- μ m nichrome monopolar stimulating electrodes were separately positioned in the LPP and MPP. In the opposite hemisphere of 12 of the rats, a control stimulating electrode was positioned in either the LPP or MPP, or a position which included both. These pathways were identified by the characteristic shape and latency of their evoked field potentials recorded in the hilus (Fig. 1*a, b*)¹⁴. The slope of the rising phase of the positive synaptic wave (e.p.s.p.) was used as the measure of synaptic strength. Control hemisphere responses over time were taken as an index of the animal's general state and provided a baseline for evaluating changes in the responses of the experimental hemisphere.

In all animals, convergence of the stimulated LPP and MPP fibres onto a common set of granule cells was verified by spatio-temporal summation tests¹⁴. In all cases, combined stimulation of the MPP and LPP produced a population spike which exceeded the criterion of three times the spike observed after linear summation of the potentials produced by each pathway alone. The synaptic strength of each pathway was evaluated by delivering diphasic test pulses of constant intensity at 0.2 Hz while the half-wave duration was altered in 16 steps over 10–250 μ s in an ascending-descending sequence. The relationship between e.p.s.p. amplitude and stimulus intensity (for example, Fig. 1*g*) will be referred to as the synaptic input/output (S-I/O) curve. Four sets of high-frequency trains (each set containing 4 trains at 1 Hz and each train containing 10 pulses of 250 μ s duration at 400 Hz) were applied at 10-min intervals to either the MPP ($n = 8$) or LPP ($n = 8$) of the experimental hemisphere. In some animals (7/16) not exhibiting robust LTP of the e.p.s.p., 5–10 additional trains (250 Hz, 320 ms) were given at 8-min intervals. Typically, the extra trains did not produce additional LTP. S-I/O curves were examined in all pathways before and 15 min following these tetani and in 10 animals were examined again 3 h post-tetanus. Finally, four sets of high-frequency trains were delivered to the previously untetanized pathway of the experimental hemisphere, followed 15 min later by another set of S-I/O curves in all pathways. S-I/O data were analysed by calculating the slope and x -intercept of the linear regression of e.p.s.p. amplitude versus the log of the stimulus intensity for those intensities above the e.p.s.p. threshold.

Tetanic stimulation of the MPP resulted in a large and lasting increase in the e.p.s.p. of the MPP (Fig. 1*c, g*). Tetanic stimulation of the LPP, however, resulted in considerably less, if any, LTP (Fig. 1*e, j*). In both conditions there was a statistically significant mean decrease of 15–20% in the e.p.s.p. amplitude of the untetanized pathway (left half of Table 1). The degree of depression was not related to the degree of convergence of the two pathways. In 7/10 animals tested, the heterosynaptic depression was still present 3 h post-tetanus, the longest interval tested. No significant correlations (r range: 0.17–0.54) were found between the magnitudes of the homosynaptic effects and the magnitudes of the heterosynaptic effects of the high-frequency stimulation. The lack of correlation is emphasized by the fact that in several animals LPP trains produced MPP

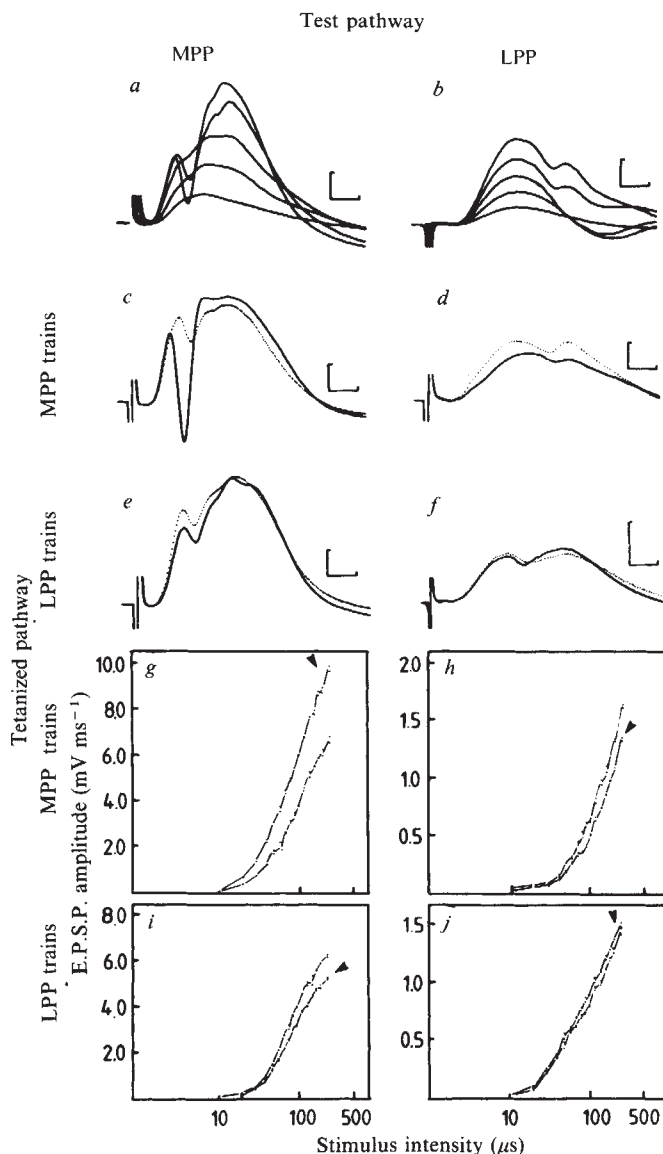


Fig. 1 Homosynaptic and heterosynaptic effects of MPP or LPP tetanization. Data from MPP S-I/O curves are shown in the left-hand column and data from LPP S-I/O curves are shown in the right-hand column. *a, b*, Typical responses to five different stimulus intensities recorded from a single animal before high-frequency stimulation. *c-f*, Single evoked responses generated by maximal intensity stimuli before and after MPP trains (*c, d*) or LPP trains (*e, f*). Dotted waveforms are responses recorded immediately before tetanization; solid waveforms are responses recorded 3 h after tetanization. Calibration bars: 1 mV, 3 ms, positive up. *g-j*, Mean synaptic I-O curves generated before and 15 min after MPP trains (*g, h*) or LPP trains (*i, j*). Arrows identify curves obtained after tetanization. An overall analysis of variance on the regression slopes indicates a significant interaction of pathway tested by temporal order of I/O testing ($F = 16.1$, d.f. = 3/84, $P < 0.001$). This result reflects the robust homosynaptic LTP produced in the MPP (*g*) and the small homosynaptic changes occurring after LPP tetanization (*j*). To test for heterosynaptic effects, a two-way analysis of covariance was performed on log-transformed data. There was a significant heterosynaptic depression of the slope of the S-I/O curves in both conditions (*h, i*), with no interaction ($F = 14.9$, d.f. = 1/13, $P < 0.01$).

depression (Table 1) in the absence of LTP of the LPP (Fig. 1*i, j*) and that in one animal MPP trains produced LPP depression but no MPP LTP. Furthermore, some animals exhibited increased heterosynaptic depression following repeated sets of trains without showing additional LTP.

The depression suffered by a pathway did not alter its potential for subsequent LTP, but prior tetanization of that pathway did

Table 1 Perforant path tetanization depresses heterosynaptic e.p.s.p.s

Prior history of tetanization	None	None	MPP	LPP
Pathway most recently tetanized	LPP	MPP	LPP	MPP
Pathway being tested	MPP	LPP	MPP	LPP
Synaptic I/O regression slope (mean % change from pre-tetanus value)	-14.4*	-20.5*	+3.1	-0.6

* $P < 0.05$, one-way analysis of covariance.

protect it from subsequent depression (right half of Table 1). This protection did not depend on the amount of prior LTP nor on the amount of LTP induced in the other pathway, and stands in contrast to the phenomenon of reversible potentiation-depression described for the crossed perforant path^{15,16}.

Previous studies in both CA1 and DG of the hippocampus have indicated that LTP is specific to the synapses activated by the tetanic stimulation and that heterosynaptic depression is typically short-lived. Our data, while confirming the synaptic specificity of LTP, demonstrate that perforant path tetanization

can depress synaptic function of converging untetanized afferents for periods exceeding 3 h. Many aspects of this long-term depression remain to be elucidated, including the duration and synaptic specificity of the effect. Given that in 3/8 animals LPP trains produced decreases in LPP synaptic strength, the depression may be nonspecific, occurring in both tetanized and untetanized pathways but usually being masked in the stimulated pathway by concurrent LTP. If so, such nonspecific depression must have maximized easily, because a second series of trains on a separate pathway failed to produce reductions in synaptic strength in either pathway. Regardless of the cellular explanation, the functional effect of heterosynaptic depression is complementary to that of LTP, that is, enhancing the relative strength of the recently active synapses, but in this case at the expense of other synapses not protected by previous LTP.

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Intracellular injections of EGTA block induction of hippocampal long-term potentiation

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Hippocampal long-term potentiation (LTP) is a remarkably stable facilitation of synaptic responses resulting from very brief trains of high-frequency stimulation^{1,2}. Because of its persistence and modest induction conditions, LTP represents a promising candidate for a substrate of memory. Some progress has been made in localizing the changes responsible for the effect; for example, it has been shown that LTP is not accompanied by changes in the fibre volleys of the test afferents³ or by generalized alterations of the dendrites of their target cells⁴. However, it is unknown whether the potentiation is due to pre- or postsynaptic changes and there is evidence in favour of each (for example, see refs 5, 6). We now report that intracellular injections of the calcium chelator EGTA block the development of LTP. These results strongly suggest that LTP is caused by a modification of the postsynaptic neurone and that its induction depends on the level of free calcium.

Slices of rat hippocampus were prepared and maintained using methods described elsewhere⁷. A stimulating electrode was placed in the stratum radiatum to activate the Schaffer-commissural fibres and an extracellular recording electrode was lowered into the apical dendritic zone of field CA1 or, in a few cases, into the pyramidal cell body layer. An intracellular electrode filled with 4 M potassium acetate (control) or potassium acetate plus 0.2-0.5 M potassium-EGTA (80-200 MΩ impedance) was then placed into the cell body layer of CA1 and attempts made to penetrate neurones. Only cells having a resting membrane potential of ≥ 55 mV ($\bar{x} \pm$ s.e.m. 66.8 ± 1.3 mV), a spike of

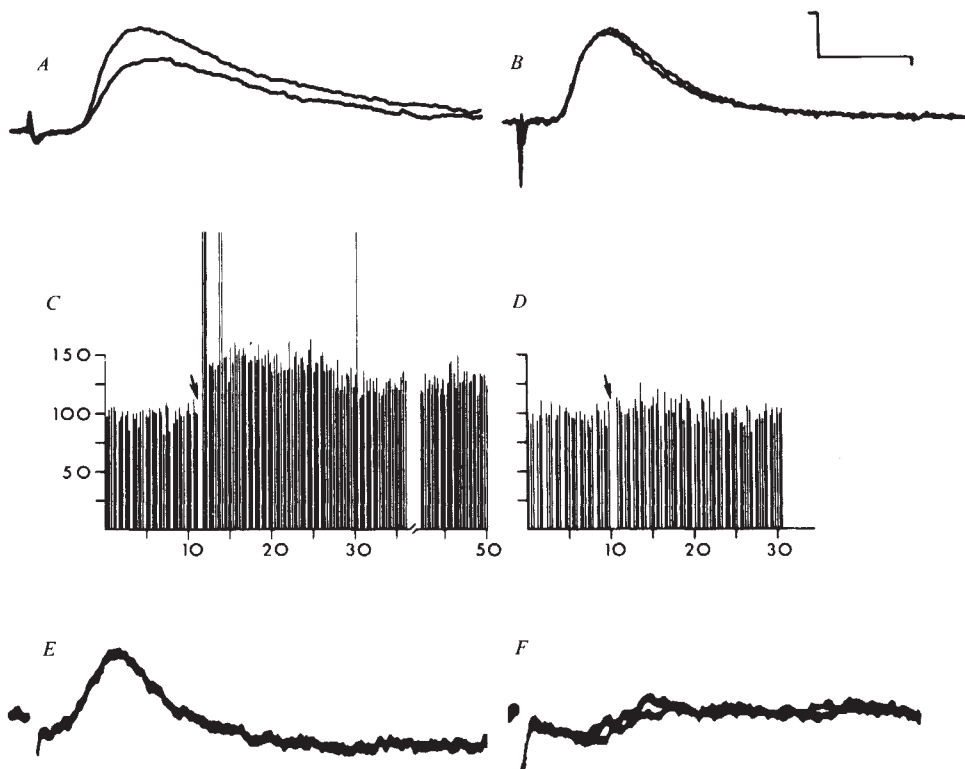
≥ 60 mV, and a membrane impedance of ≥ 15 MΩ ($\bar{x} \pm$ s.e.m. 30.6 ± 2.4 MΩ) were used. In a few cases, a small hyperpolarizing current was used to increase the size of the excitatory postsynaptic potential (e.p.s.p.). If a satisfactory impalement was achieved, stimulation pulses were delivered at a rate of one per 10 s to the Schaffer-commissural fibres. The stimulation current (20-70 μ A) was adjusted to a level at which the e.p.s.p. amplitude could be reliably measured but was sub-threshold for spike discharge, and at which the dendritic field potential was not maximal.

The following measurements were made by computer following each stimulus: (1) rise time and amplitude of the e.p.s.p.; (2) resting membrane potential; and (3) slope and amplitude of the extracellular dendritic field potential (or amplitude of the population spike). If these measurements proved stable, 5-7 min of 200-ms 0.5 nA pulses given at 2 per s were applied to EGTA electrodes and several of the regular electrodes. Following this, testing was resumed for ≥ 10 min and, if the responses proved stable, a series of high-frequency trains were applied to the stimulation electrode. These consisted of five 300 s⁻¹ bursts, each of 35 ms duration, or 100 s⁻¹ bursts each of 300 ms duration. Testing was resumed within 15 s and recordings taken for 10-90 min, depending on the stability of the neurone. Finally, the recording pipette was withdrawn from the neurone and extracellular responses collected.

Intracellular data were used if the resting membrane potential did not vary by more than 2.5 mV from the control period to at least 10 min after the high-frequency stimulation trains. Only intracellular recordings from slices which exhibited extracellular LTP were included in the study. LTP was defined as a non-decremental increase of greater than 10% of the slope and amplitude of the dendritic response recorded by the extracellular electrode during the period 5-15 min after the high-frequency stimulation. If an extracellular response was reliably detected by the intracellular electrode after leaving the cell, it was averaged and subtracted from the stored e.p.s.ps.

Thirty-three cells were studied with regular electrodes from slices with extracellular LTP (mean of $28.5 \pm 2.2\%$ increase of field potential slope). Figure 1A, C illustrates one of these. Five

Fig. 1 *A*, Intracellular recording with a regular electrode. Each trace is the average of six successive responses 10 s apart collected immediately before and 15 min after five bursts of 300 s^{-1} stimulation, each lasting 35 ms, delivered through a bipolar electrode in the trajectory of the Schaffer-commissural fibres. Calibration bars: 5 mV and 5 ms for this and subsequent panels. *B*, Intracellular recording with an EGTA-filled electrode. Averaged responses to six single-pulse stimulations collected immediately before and 15 min after four high-frequency stimulation trains are superimposed. *C*, Per cent change in amplitude of the e.p.s.p. after high-frequency stimulation (arrow) for the cell illustrated in *A*. The x-axis is time in minutes. Each line represents a single response and the average amplitude for the control period is expressed as 100%. The high-amplitude responses are spikes which have been chopped in preparation of the figure. *D*, Amplitude of individual e.p.s.ps expressed as percentage of the averaged responses collected before high-frequency stimulation for the cell described in *B*. EGTA was injected before collecting control data. *E*, Intracellular recording from a neurone with an EGTA-filled electrode. Calibration as in *A*. *F*, Recording as in *E* but after withdrawal from the cell.



of the neurones recorded with regular electrodes did not show increased e.p.s.ps after the high-frequency train, five spiked to each single stimulus pulse, and 23 exhibited stable potentiation of the type illustrated in Fig. 1. Thus, intracellular LTP was obtained in 28 of 33 cells. Table 1 summarizes the mean percentage increase in e.p.s.p. for the cells ($n=28$) which did not spike. Twenty-six cells were studied with EGTA-filled electrodes from slices which exhibited LTP (mean increase of $28.3 \pm 4.4\%$ in slope of dendritic field potential); 20 did not exhibit evidence of LTP (Fig. 1*B*, *D*), four showed at least a 10% increase in e.p.s.p., while two neurones spiked to single stimula-

tion pulses after high-frequency stimulation. Thus, LTP was obtained in only six of 26 neurones recorded with EGTA-filled electrodes. Table 1 summarizes per cent change in the 24 cases in which e.p.s.ps could be recorded following high-frequency stimulation.

In agreement with previous reports^{8,9}, EGTA injections had no detectable effect on resting membrane potential or membrane impedance. As seen in Table 1, the mean e.p.s.p. amplitude before high-frequency stimulation was comparable in the two groups. It is worth noting that similar dendritic field potential amplitudes were obtained in both groups (control, 2.2 ± 0.1 mV;

Fig. 2 *A*, Paired-pulse facilitation of the e.p.s.p. in a cell recorded with an EGTA-filled electrode. Same cell as shown in Fig. 1*E*. Calibration bars: 10 mV and 20 ms. *B*, *C*, Recordings from slices in medium containing $20\text{ }\mu\text{M}$ picrotoxin to prevent γ -aminobutyric acid mediated i.p.s.ps. The left record of each pair is the response photographed from the oscilloscope at low gain and fast sweep speed (calibration bars: 25 mV and 25 ms); the right-hand record is the same response as a digitized record displayed at high gain and slow sweep speed (calibration bars: 5 mV and 1 s). *B*, *i*, Depolarizing current-induced burst with associated slow AHP in a cell recorded with a regular electrode. *B*, *ii*, Similar burst induced in a cell recorded with an EGTA-filled electrode, but the AHP is absent. *C*, *i*, Repetitive firing induced by s. radiatum stimulation in the same cell shown in *B*, *i* and associated AHP. *C*, *ii*, Similar firing evoked in the EGTA-filled cell of *B*, *ii*; AHP is not blocked. Resting membrane potential of control cell (regular electrode), -60 mV; that of EGTA-filled cell, -58 mV.

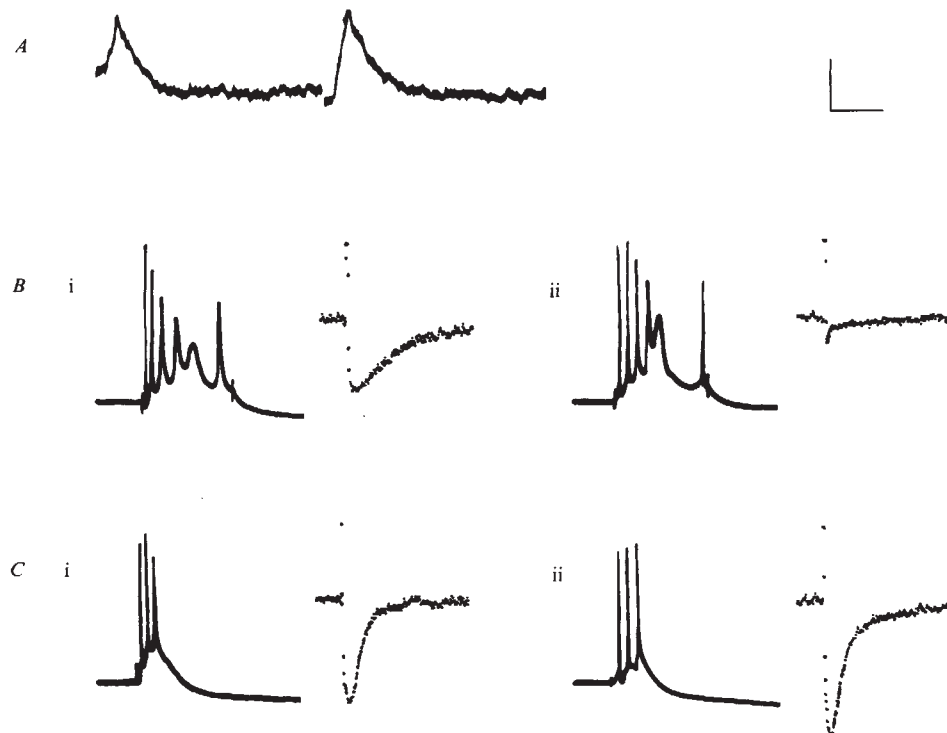


Table 1 e.p.s.p. amplitudes of cells which did not spike to single pulse stimulation after induction of extracellular long-term potentiation

	e.p.s.p. amplitude before high-frequency stimulation (mV)	Time (min)		
		5	10	15
Control	7.0 ± 0.6	+30 ± 5%	+30 ± 4%	+28 ± 4%
EGTA	7.1 ± 0.6	+8 ± 3%*	+1 ± 3%*	+5 ± 4%*

The e.p.s.p. amplitudes (mean ± s.e.m.) for 28 of 33 cells recorded with regular electrodes (control) and 24 of 26 cells recorded with EGTA-filled electrodes.

* $P < 0.001$.

EGTA, 2.1 ± 0.1 mV), indicating that the stimulation current used to obtain similar sized e.p.s.ps in the two groups was not different. Furthermore, excellent paired-pulse facilitation was found in these cells (Fig. 2A) and they typically spiked to single pulse stimulation for 30–45 s after high-frequency stimulation, indicating post-tetanic potentiation (PTP) was present. Additional evidence of the selective action of EGTA has been documented in studies of burst after-hyperpolarizations (AHPs)^{8,9}. We have found that in slices incubated in medium containing 20 μ M picrotoxin (used to block the inhibitory postsynaptic potential (i.p.s.p.) which obscures the initial part of the AHP), EGTA blocks the AHP following bursts induced by depolarizing current injection, but does not block the AHP following repetitive firing induced by stratum radiatum stimulation (Fig. 2B, C). As other evidence indicates that the depolarization-induced AHP is a Ca^{2+} -dependent potential^{8–11}, we suggest that the only effect of EGTA in these neurones was on Ca^{2+} -mediated processes.

These results indicate that selective manipulation of the post-synaptic neurone blocks the induction of long-term potentiation. As EGTA did not produce evident disturbances in several physiological parameters in our experiments or those reported by others^{8,9}, it is reasonable to assume that it interfered with the induction of LTP via its known actions as a calcium buffer. Therefore, the present findings strongly support the hypothesis that LTP is due to a calcium-stimulated modification of the postsynaptic neurone¹². Previous studies have established that low levels of calcium cause an increase in glutamate binding sites in purified membranes from hippocampus¹³, apparently by activating a calcium-sensitive proteinase^{14–16}. Furthermore, LTP is correlated with an increase in glutamate binding to hippocampal membranes^{17,18}. We propose that EGTA, by buffering intracellular calcium, prevents the activation of enzymatic machinery controlling postsynaptic receptors and thereby blocks the development of LTP.

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Novel C-terminally amidated opioid peptide in human pheochromocytoma tumour

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As has often been observed in hypothalamic releasing factors and gastrointestinal hormones, the carboxy-terminal amide structure is a unique feature of peptides exhibiting hormonal or physiological activities. Although a variety of opioid peptides have hitherto been identified^{1–8}, such a C-terminal amidated species has never before been discovered in mammals. Here we present the first identification of a novel opioid octapeptide with a C-terminal amide structure, henceforth designated as 'adrenorphin', in human pheochromocytoma tumour derived from adrenal medulla. The complete amino acid sequence of adrenorphin was determined by microsequencing and corresponds to the sequence of the first eight amino acids of peptide E which is derived from proenkephalin A. Adrenorphin has also been identified chromatographically in normal human and bovine adrenal medulla. Adrenorphin exhibits potent opioid activity in guinea pig ileum assay, suggesting a specialized physiological function.

Adrenorphin was purified from human pheochromocytoma using a technique similar to that used previously in the isolation of opioid peptides such as BAM-12P from bovine adrenomedullary gland³. A portion corresponding to the lower molecular weight ($M_r < 2,000$) that was separated from acid extracts of human pheochromocytoma by gel filtration on Sephadex G-50 was treated batchwise with CM-cellulose in a buffer of 10 mM ammonium formate (pH 6.6). After washing the resin with the same buffer, the basic peptides adsorbed on the column were eluted with 1 M formic acid and pooled. The basic peptide pool thus obtained was then subjected to HPLC on a reverse-phase column of TSK LS-410 ODS-SIL (Fig. 1). An aliquot of each fraction was trypsinized and then generation of [Arg⁶]-enkephalin was analysed by radioimmunoassay utilizing anti-[Arg⁶]-Leu-enkephalin antiserum, having 5% cross-reactivity with [Arg⁶]-Met-enkephalin⁹. As seen in Fig. 1, five immunoreactive peaks (A–E) were obtained. The present purification of adrenorphin is concerned with peak B, which was eluted close to the position corresponding to the authentic BAM-12P.

The subsequent reverse-phase HPLC of peak B on a different column of μ -Bondapak C-18, yielded adrenorphin as an unidentified peptide peak that was eluted slightly ahead of the standard BAM-12P. Rechromatography afforded purified adrenorphin, as shown in Fig. 2a. Only a tyrosine residue was identified by dansylation as the amino-terminal residue of adrenorphin thus purified, confirming its homogeneity. Attempted C-terminal analyses of the peptide utilizing carboxypeptidase A and B revealed that the C-terminus of adrenorphin is blocked.

On hydrolysis with 6 M HCl, adrenorphin afforded the following amino acid composition: Gly_{2.2}, Val_{1.0}, Met_{0.9}, Tyr_{1.0}, Phe_{1.0}, Arg_{2.0}, suggesting its octapeptide structure. Based on quantitative amino acid analysis of adrenorphin, it is estimated that 20 nmol of the pure peptide were isolated from 42.6 g of the tumour. Trypsinization of the peptide generated [Arg⁶]-Met-enkephalin, which was identified with the standard specimen by chromatographic comparison on reverse-phase HPLC (Fig. 3). The primary structure of adrenorphin was determined by stepwise Edman-dansyl sequencing of the native peptide³. The complete amino acid sequence of adrenorphin thus ascertained is H-Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH₂. The presence of C-terminal valine amide was verified by thermolytic digestion, followed by dansylation in a manner similar to that described by Tatamoto *et al.*¹⁰.

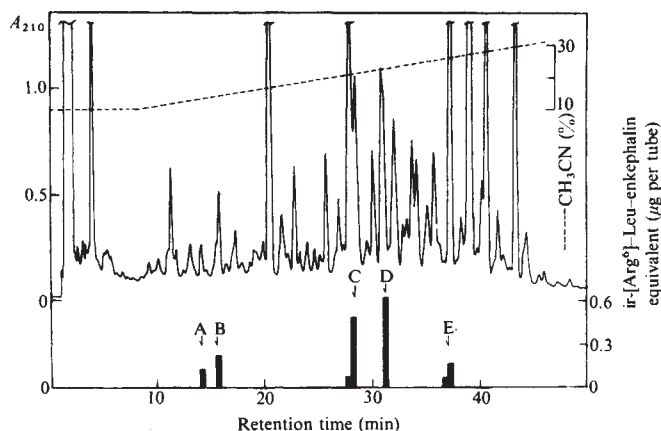


Fig. 1 Reverse-phase HPLC of the small basic peptide fraction obtained from human pheochromocytoma. An aliquot taken from each fraction tube was trypsinized at pH 8.0 and the $[\text{Arg}^6]$ -enkephalin generated was assayed by radioimmunoassay with an antiserum against $[\text{Arg}^6]$ -Leu-enkephalin⁹. Immunoreactivity represented by a black bar is expressed as $[\text{Arg}^6]$ -Leu-enkephalin equivalent. Arrows indicate elution positions of the standard opioid peptides identified in bovine adrenal medulla: A, $[\text{Arg}^6]$ -Met-enkephalin; B, BAM-12P (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu); C, $[\text{Arg}^6]$, Gly⁷, Leu⁸-Met-enkephalin; D, $[\text{Arg}^6]$, Phe⁷-Met-enkephalin; E, BAM-18P (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu-Trp-Trp-Met-Asp-Tyr-Gln). **Methods:** A human pheochromocytoma tumour (42.6 g) resected at surgery was cooled on ice immediately after removal. Diced pieces of the tumour were boiled for 10 min in 5 volumes of 1 M acetic acid containing 20 mM HCl to inactivate intrinsic proteases, and were then homogenized. The supernatant of the extracts was subjected to gel filtration on a column of Sephadex G-50 (3×150 cm) to collect the fraction corresponding to the lower molecular weight ($M_r < 2,000$). The pooled fraction was passed through a Sep-Pak cartridge (Waters Associates). The dried material obtained was dissolved in a buffer of 10 mM ammonium formate (pH 6.6) and adsorbed on a column of CM-cellulose (1.0×5 cm). After washing the resin thoroughly with the same buffer to remove acidic and neutral peptides, the fraction containing basic peptides was eluted with 1 M formic acid and pooled. After lyophilization, the peptides thus obtained were applied to HPLC on a reverse-phase column (0.4×25 cm) of TSK LS-410 ODS-SIL (Toyosoda). The column was eluted with a linear gradient (80 min) of CH_3CN concentration from 10 to 50% in 50 mM potassium phosphate buffer (pH 2.0) at a flow rate of 2 ml min^{-1} . The effluent was monitored by absorbance at 210 nm and collected in a small test tube every 30 s.

For structural confirmation, the octapeptide amide according to the adrenorphin structure determined above was synthesized by solid phase techniques, conducted on a *p*-methyl-benzhydrylamine resin. Amino acid analysis and microsequencing confirmed the correct synthesis of adrenorphin. Des-amido-adrenorphin (adrenorphin-OH) having a free carboxy-terminus was also synthesized. As shown in Fig. 2b, c, natural and synthetic adrenorphin migrate together on both reverse-phase and cation-exchange HPLC, and they are well separated from synthetic adrenorphin-OH by cation-exchange HPLC. This confirms that adrenorphin was isolated in an amide form, and not in a free carboxyl form. There is also evidence that natural adrenorphin undergoes tryptic digestion in exactly the same manner as the synthetic specimen (Fig. 3). Furthermore, natural adrenorphin shows opioid activity in guinea pig ileum assay with the same potency as the synthetic specimen. For these reasons, the complete structure of adrenorphin that terminates in valine amide is definitely established. Thus, adrenorphin is proven to be the first opioid peptide ever identified as a C-terminal amidated form in mammals.

The sequence corresponding to adrenorphin thus determined is found to be present within human (and also bovine) preproenkephalin A at positions 210–217, suggesting that adrenorphin is derived from this precursor^{11,12}. Moreover, the adrenorphin

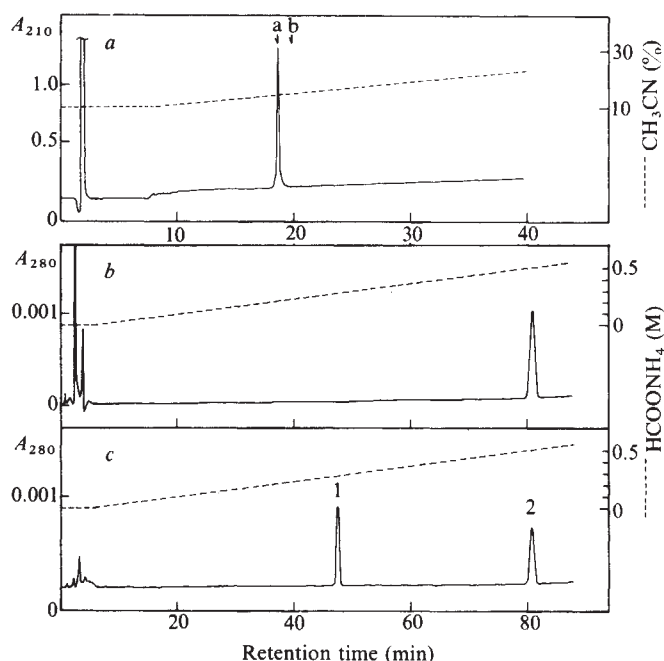


Fig. 2 a, Final purification of adrenorphin by reverse-phase HPLC. The material obtained from the immunoreactive peak B in Fig. 1 was applied to HPLC on a column (0.39×30 cm) of μ -Bondapak C-18 (Waters Associates). Elution was carried out with a linear gradient (40 min) from a solvent of 12.2 mM ammonium formate (pH 4.0)- CH_3CN (90:10) to 20 mM ammonium formate (pH 4.0)- CH_3CN (50:50) at a flow rate of 2 ml min^{-1} . An aliquot of each fraction was digested with trypsin and subjected to radioimmunoassay for $[\text{Arg}^6]$ -enkephalins. The major immunoreactive peak eluted slightly ahead of BAM-12P as above. Adrenorphin was purified as a single immunoreactive peak, confirming its purity, and was used for structural analyses. Arrows indicate elution positions of synthetic adrenorphin (a) and BAM-12P (b). Natural and synthetic adrenorphin migrated together under these conditions. b, Cation-exchange HPLC of natural adrenorphin. Natural adrenorphin (2 nmol) was applied to a column (0.4×30 cm) of TSK IEX-530 CM (Toyosoda), pre-equilibrated with a buffer of 0.01 M ammonium formate (pH 6.6)- CH_3CN (90:10). Elution was carried out with a linear gradient (160 min) from the starting buffer to 1.0 M ammonium formate (pH 6.6)- CH_3CN (90:10). Flow rate was 1.0 ml min^{-1} . The effluent was monitored by measuring absorbance at 280 nm. Natural and synthetic adrenorphin migrated together on this HPLC system. c, Cation-exchange HPLC of synthetic adrenorphin-OH (1) and adrenorphin (2). The respective peptides (2 nmol each) were applied to a TSK IEX-530 CM column under the same conditions as for b.

sequence in the precursor is followed by a glycine residue (Fig. 4) which is essential for C-terminal amidation¹³, serving as a nitrogen donor to amidate the preceding valine residue. However, unlike the precursors of the other C-terminal amidated hormones^{14–16}, where the glycine residue is followed by a unique signal of paired basic residues, the glycine residue attached to the adrenorphin unit is connected to the sequence of -Arg-Pro-, which has not so far been regarded as a typical processing signal^{12,17}. This is why the natural occurrence of adrenorphin has not been anticipated from the structural basis of the precursor. However, note that PH-8P (dynorphin [1–8]), a predominant opioid peptide in brain^{5,18} is also followed by the sequence -Arg-Pro- in preproenkephalin B¹⁹, implying that processing at the signal of -Arg-Pro- results in the formation of PH-8P. (Fig. 4) Accordingly, adrenorphin is thought to be programmed to be processed out of the precursor in a C-terminal amidated form.

Adrenorphin exhibits remarkable opioid activity in guinea-pig ileum assay²⁰. Adrenorphin (IC_{50} : 1.7 nM) is 15 times and 11

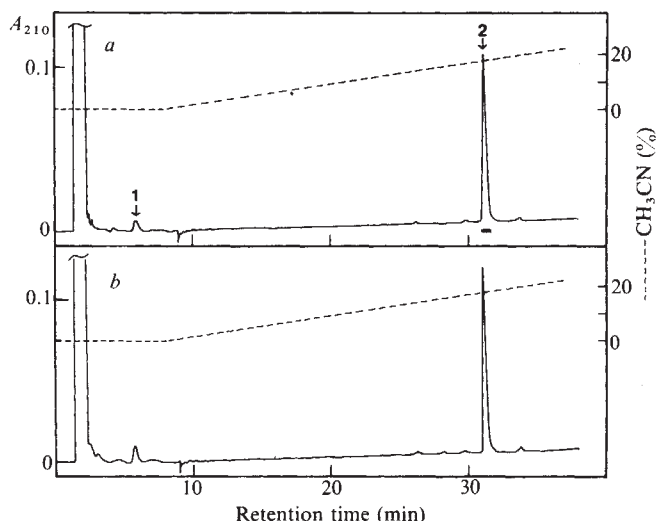


Fig. 3 Reverse-phase HPLC of the tryptic digests of natural (a) and synthetic (b) adrenorphin. The peptide (2.0 nmol) was digested with 0.1 μ g of TPCCK-treated trypsin (Worthington) in 50 μ l of 0.1 M sodium bicarbonate (pH 8.0) at 37 $^{\circ}$ C for 2 h. After acidification with 1 M formic acid, the reaction mixture was directly loaded to a TSK LS-410 ODS-SIL column (0.4 $\times$ 25 cm). Elution was carried out with a linear gradient (80 min) of a concentration of CH₃CN from 0 to 60% in 0.1% tetrafluoroacetate solution. Flow rate was 2 ml min⁻¹. The effluent was monitored by absorbance at 210 nm. a, The tryptic digest of natural adrenorphin afforded two peptide peaks, (1) and (2), which were identified as Arg-Val-NH₂ (1) and Tyr-Gly-Gly-Phe-Met-Arg (2), respectively. Black bar indicates immunoreactivity for [Arg⁶]-enkephalin. b, Synthetic adrenorphin underwent trypsinization in exactly the same manner as natural adrenorphin.

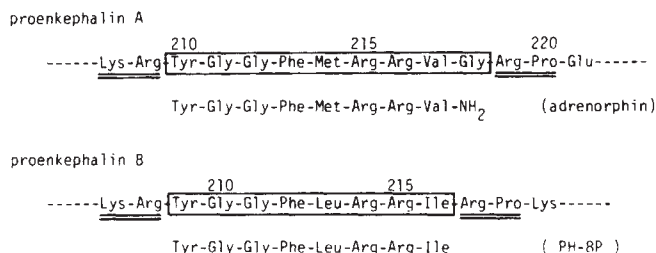


Fig. 4 Location of adrenorphin and PH-8P in their respective precursors. Residue numbers are taken from the sequences of human preproenkephalin A¹¹ and porcine preproenkephalin B¹⁹. The sequences corresponding to adrenorphin and PH-8P are boxed and the flanking residues are underlined. Natural occurrence of adrenorphin and PH-8P indicates that a sequence of -Arg-Pro- is likely a processing signal.

times more potent than Met-enkephalin and synthetic adrenorphin-OH, respectively. Interestingly, BAM-12P, the C-terminal extended peptide of adrenorphin, is only one-fifth as potent as adrenorphin. Assuming that the enkephalin sequence represents the 'message' and the specific C-terminal extension the 'address'²¹, the unique C-terminal structure of adrenorphin may suggest the presence of a specific receptor to adrenorphin and a specialized function.

Note added in proof: Weber *et al.* have recently described an identical peptide from bovine brain²².

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Basolateral KCl co-transport in a NaCl-absorbing epithelium

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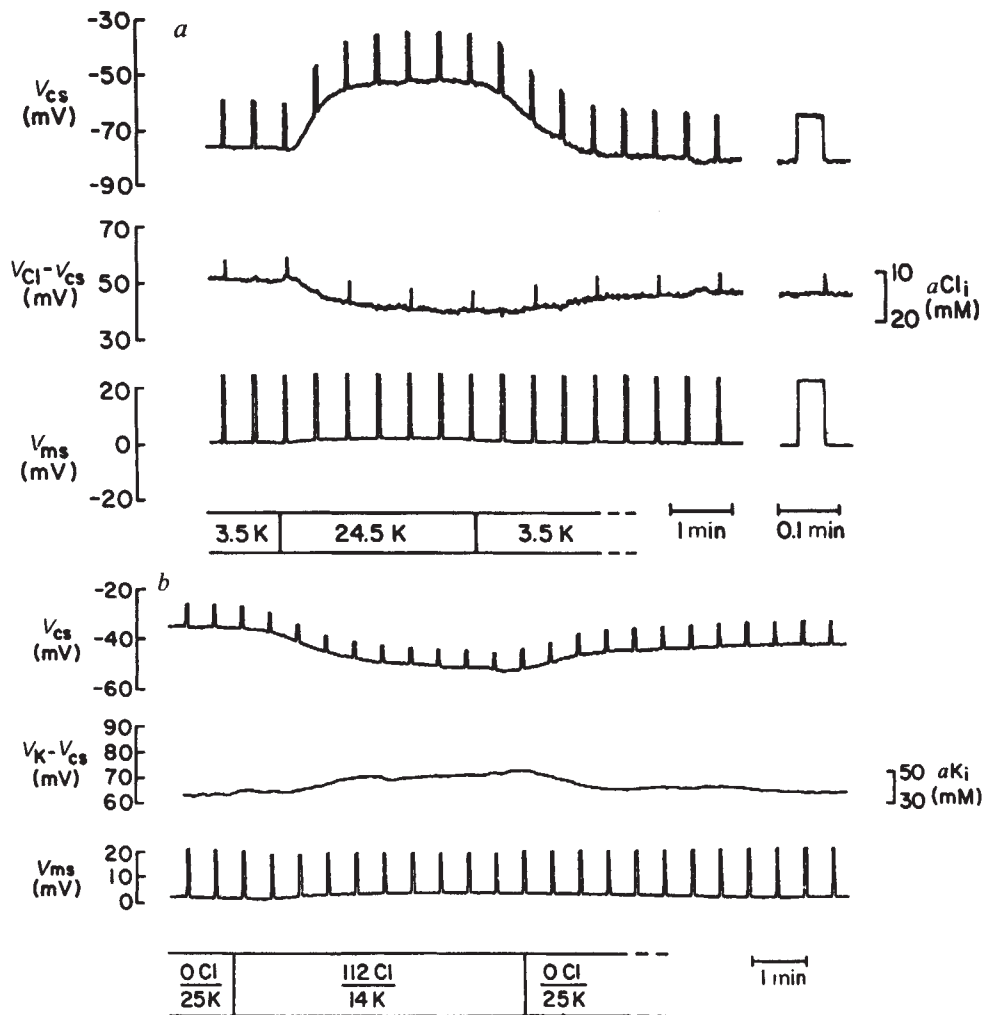
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In NaCl-absorbing epithelia such as proximal renal tubule, small intestine and gallbladder, Na⁺-dependent Cl⁻ entry across the luminal membrane is an electroneutral transport process that has been attributed to Na-Cl symport¹⁻⁴, Na-K-Cl symport^{5,6}, or a double (Na-H, Cl-HCO₃) antiport⁷⁻⁹. At the basolateral (antiluminal) membrane, Na⁺ extrusion can be attributed to the Na⁺-K⁺ pump, and Cl⁻ transport could be explained in principle by electrodiffusion since the intracellular Cl⁻ activity is higher than predicted for equilibrium distribution¹⁻³. However, in *Necturus* gallbladder¹⁰, as in other epithelia¹¹⁻¹⁴, the electrodiffusional Cl⁻ permeability of the membrane (P_{Cl}) is too low to account for the transepithelial Cl⁻ transport rate¹⁰. Because K⁺ is at a higher chemical potential in the cell than in the extracellular fluid³, and because serosal Cl⁻ substitutions have only small effects on membrane potential¹⁰, the hypothesis of carrier-mediated electroneutral KCl co-transport was proposed¹⁰. The experiments reported here were designed to test this hypothesis in *Necturus* gallbladder epithelium. Intracellular Cl⁻ and K⁺ activities (a_{Cl} , a_{K}) were measured with ion-sensitive intracellular microelectrodes before, during and after ionic substitutions of the serosal (basolateral) bathing medium. The results demonstrate a Na⁺-independent basolateral membrane KCl symport.

Gallbladders were mounted mucosal side up in a chamber that allows for continuous separate perfusion of mucosal and serosal sides. In most experiments, the tissues were initially bathed on both sides with Na-Ringer's solution (in mM: 109.2 NaCl, 2.5 NaCl, 2.5 KCl, 1.0 K-HEPES, 2.0 CaCl₂, pH ~7.7, gassed with air). Experiments in solutions buffered with 10 mM HCO₃/1% CO₂ yielded similar results. Intracellular and transepithelial electrical measurements were carried out as described before^{3,15}. Cell-membrane potentials and intracellular ionic activities were determined from simultaneous impalements of two neighbouring epithelial cells with a conventional microelectrode (filled with 3 M or 0.5 M KCl) and a K⁺- or Cl⁻-sensitive microelectrode. Continuous records before, during and after ionic substitutions were obtained. The impalements were validated by the criteria previously described (ref. 3, see also Fig. 1).

The three main experimental procedures were: (1) measurement of the effects of increasing serosal K⁺ concentration ([K]) on a_{Cl} ; if a KCl symport exists, a_{Cl} should rise. (2) Determination of the effects of simultaneous changes of serosal [K] and [Cl] on a_{K} ; in the case of a KCl symport, a moderate reduction of [K] simultaneous with a large increase of [Cl] could cause an

Fig. 1 *a*, Effects of an increase in serosal [K] (millimolar concentrations indicated at the bottom) on basolateral cell membrane potential (V_{cs} , top trace), intracellular Cl^- activity (aCl_i , middle trace) and transepithelial electrical potential (V_{ms} , bottom trace). The aCl_i record is the difference between the cell membrane potentials measured with a Cl^- -sensitive and a conventional (3 M KCl) microelectrode. The records start with the microelectrodes inside cells in the same area of the tissue. The voltage deflections at 30-s intervals are the result of transepithelial current pulses of $\sim 50 \mu A cm^{-2}$. After correction for the voltage drops in the subepithelial tissue and the bathing solutions, the transepithelial resistance and the apparent ratio of cell membrane resistances can be calculated. Impalements with conventional and ion-sensitive microelectrodes were validated as described before³. As shown at the end of the trace, the steady-state voltage deflections produced by the transepithelial current pulses were equal in the two impaled cells. The brief deflections produced in the differential trace by some of the pulses are caused by the different time constants of the two microelectrodes. The increase in serosal [K] caused a reduction of V_{cs} , a slight change of V_{ms} in the mucosa-positive direction, and an increase in aCl_i . The changes in V_{cs} and V_{ms} are due to the high relative K^+ permeability of both basolateral membrane and paracellular pathway^{17,18}. All effects were reversible. *b*, Effects of simultaneous changes in serosal [Cl] and [K] on membrane potentials and aK_i (middle trace). The tissue was exposed to a nominally K-free mucosal bathing medium. [Cl] and [K] were changed on the serosal side only as indicated at the bottom. A reduction of [K] simultaneous with an increase in [Cl] caused reversible increases in V_{cs} and aK_i . The change in the V_{ms} trace is mostly the result of a change in liquid-junction potential.



elevation of aK_i ; if K^+ were transported only by the Na^+-K^+ pump and an electrodiffusional pathway, the reduction of serosal [K] would result in a fall of aK_i by decreased pumping and/or increased net electrodiffusional efflux. (3) The Na^+ -dependency of KCl co-transport was tested: aCl_i was measured before and after removing Na^+ from the serosal side and the effect of elevating serosal [K] was compared in the two conditions.

The effect of changing serosal [K] is shown in Fig. 1*a* and summarized in Fig. 2. Increasing serosal [K] caused significant increases of aCl_i (Fig. 2*a*), as previously shown by Corcia and Armstrong¹⁶. This effect does not depend on the presence of Cl^- in the mucosal bathing medium (Fig. 2*b*). In tissues exposed to a nominally Cl^- -free mucosal bathing medium, increases in serosal [K] caused significant increases in aCl_i , although they were smaller than the analogous changes in the presence of mucosal Cl^- . In these experiments, the basolateral membrane Cl^- influx is expected to cause a much smaller change in aCl_i , because the large gradient across the luminal membrane favours efflux.

In both series of experiments, however, the observed increases of aCl_i could depend, fully or in part, on the basolateral membrane depolarization elicited by raising serosal [K]. This possibility was ruled out by two sets of experiments. First, aCl_i was measured before, during and after 5-min-long transepithelial current clamps that altered the basolateral membrane potential by 10–40 mV in either direction. No significant changes in aCl_i were observed. Second, in tissues initially exposed to a Cl^- -free

mucosal solution, [K] was elevated in the mucosal, but not in the serosal bathing medium. Because of the high paracellular ionic permeability of this epithelium and because the apical membrane is mainly K^+ permeable^{17,18}, this manoeuvre depolarizes both cell membranes (luminal and basolateral), but Cl^- entry across the luminal membrane is not possible because the external medium is Cl^- free. Hence, the possibility that basolateral membrane Cl^- entry is caused by depolarization is tested. I found that elevation of luminal [K] for 5 min did not increase aCl_i (Fig. 2*c*). Longer exposures to high- K^+ mucosal solution were not tested, since they can cause rises in basolateral [K] because of paracellular K^+ permeation¹⁷. Taken together, these results demonstrate that elevations of serosal [K] cause increases of aCl_i , and that this effect cannot be attributed to either Cl^- uptake at the apical membrane or basolateral membrane depolarization. The possibility of a reduction of cell volume in high- K^+ medium can also be ruled out, since optical measurements in similar experimental conditions show no significant cell volume changes within the duration of the experiments shown in Fig. 2 (M. Larson and K. R. Spring, personal communication).

If the results described above are in fact due to a basolateral KCl symport, changes in basolateral [Cl] should cause changes of aK_i in the same direction. Furthermore, appropriate simultaneous changes of both extracellular [K] and [Cl] could cause changes in aK_i in a direction opposite to that expected for a membrane across which K^+ can only be transported by the Na^+-K^+ pump or by electrodiffusion. In the experiments illus-

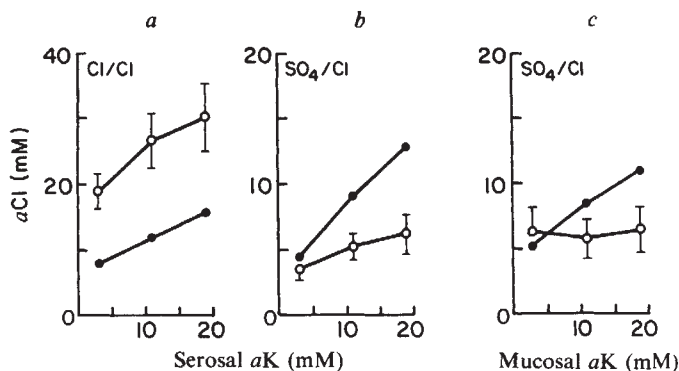


Fig. 2 Effects of changes in serosal or mucosal solution $[\text{K}]$ on intracellular Cl^- activity ($a\text{Cl}_i$). The control serosal bathing solution was Na-Ringer's (NaCl main salt) with 3.5 mM K^+ . $[\text{K}]$ was increased by isomolar Na substitution. In all three panels, the symbols at the top indicate the main anion in the mucosal and serosal side respectively. The measured intracellular Cl^- activity ($a\text{Cl}_i$; ○) and the calculated Cl^- equilibrium activity across basolateral membrane ($a\text{Cl}_i^{\text{eq}}$; ●) are plotted as functions of K^+ activity (aK) in the serosal (a, b) or mucosal side (c). The $a\text{Cl}_i$ values at high aK were measured 5 min after the substitution. Means or means $\pm$ s.e.m. are shown. Statistical comparisons were made by pair data analysis. a, During bilateral exposure to Cl^- media, increasing serosal aK from 3 to either 11 or 19 mM significantly increased $a\text{Cl}_i$ ($P < 0.01$ and $P < 0.025$ respectively, $n = 5$); V_{cs} values from low to high serosal aK were -63 ± 4 , -52 ± 4 and $-44 \pm 4 \text{ mV}$ respectively. b, In the absence of mucosal Cl^- (SO_4 replacement), $a\text{Cl}_i$ fell to a value near equilibrium across the basolateral membrane. Increasing serosal aK resulted in significant, but smaller increases of $a\text{Cl}_i$ ($P < 0.05$, $n = 6$, for the two comparisons analogous to those in a); V_{cs} values were -74 ± 5 , -56 ± 2 and $-47 \pm 2 \text{ mV}$ respectively. c, Increasing mucosal $[\text{K}]$ in the absence of Cl^- on that side had no significant effect on $a\text{Cl}_i$; V_{cs} values were -73 ± 5 , -61 ± 5 and $-52 \pm 6 \text{ mV}$ respectively.

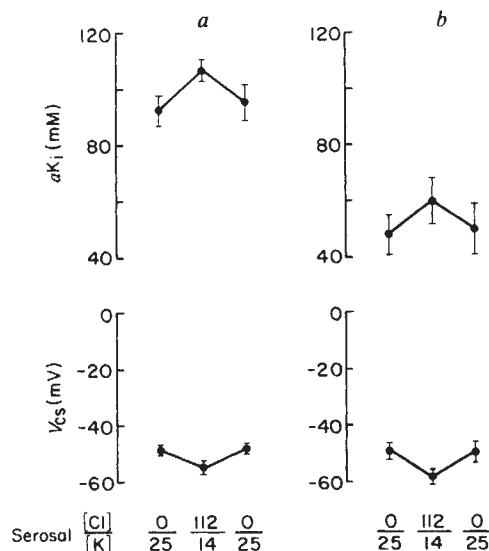


Fig. 3 Effects of simultaneous changes in serosal $[\text{Cl}]$ and $[\text{K}]$ (bottom) on aK_i and V_{cs} . Experiments with a, control mucosal bathing solution and b, nominally K-free mucosal bathing solution. Values in high-K, low-Cl medium were measured 5 min after the serosal solution substitution. In both cases, a reduction of $[\text{K}]$ simultaneous with an increase in $[\text{Cl}]$ caused significant increases of aK_i ($P < 0.025$, $n = 5$, and $P < 0.01$, $n = 6$, in a and b respectively). The basolateral membrane hyperpolarization was also statistically significant and reversible in both series of experiments. Means $\pm$ s.e.m. shown.

trated in Fig. 1b and summarized in Fig. 3, $[\text{K}]$ was reduced from 25 to 14 mM, and at the same time $[\text{Cl}]$ was elevated for a pump/leak model, aK_i rose, a result consistent with the hypothesis of KCl co-transport.

However, the result shown in Fig. 3a could be caused by K^+ entry across the luminal membrane, by increased K^+ uptake by the pump or by a decrease of cell volume without net K^+ uptake. The first of these possibilities was ruled out by the experiments shown in Fig. 3b: aK_i rose also when the serosal solution substitution was carried out in the nominal absence of K^+ in the mucosal bathing medium. The second possibility, unlikely at the high extracellular $[\text{K}]$ used in this series of experiments, was also experimentally ruled out by performing the same serosal solution substitution in tissues treated with ouabain (10^{-4} M). In these experiments, aK_i rose from 43 ± 3 to $50 \pm 7 \text{ mM}$ ($P < 0.01$, $n = 5$). The possibility of cell shrinkage when serosal $[\text{K}]$ is lowered and serosal $[\text{Cl}]$ raised was indirectly addressed by comparing the changes in $a\text{Cl}_i$ and aK_i produced by this perturbation. Both activities rose ($a\text{Cl}_i$ by $6.4 \pm 2.0 \text{ mM}$, aK_i by $8.1 \pm 2.2 \text{ mM}$; paired differences not significant; $n = 4$). As both aK_i and $a\text{Cl}_i$ rise, one can conclude that the cells gain salt, and hence swell. The similarity of the changes suggests that the entry process is electroneutral. Thus the effects of basolateral $[\text{K}]$ on $a\text{Cl}_i$ and the effects of basolateral $[\text{Cl}]$ on aK_i are consistent with the hypothesis of a KCl symport. Appropriate control experiments rule out alternative explanations.

Because of the growing body of evidence supporting Na-K-Cl co-transport in nonpolar¹⁹⁻²¹ and epithelial cells^{5-6,22-24}, this possibility was tested by comparing $a\text{Cl}_i$ values in tissues exposed first to a Na-containing basolateral solution and then to a nominally Na-free medium (tetramethylammonium substitution). In both conditions, the effect of an increase in $[\text{K}]$ on $a\text{Cl}_i$ was studied as well. The results (Fig. 4) rule out a contribution

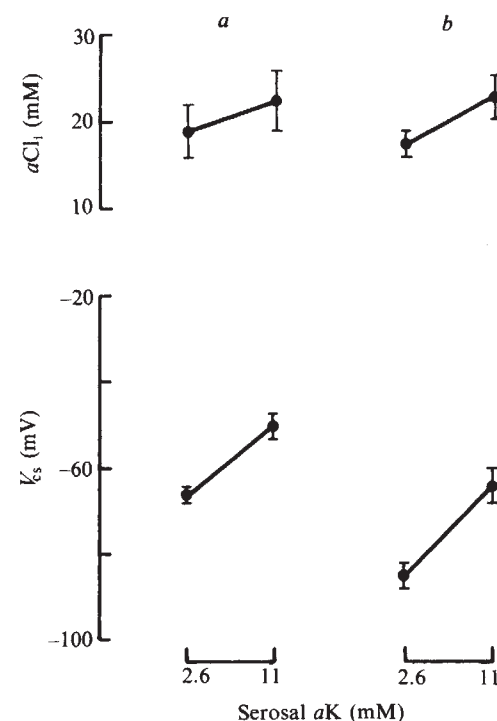


Fig. 4 Effects of increasing basolateral $[\text{K}]$ on $a\text{Cl}_i$ and V_{cs} in tissues exposed to Na-Ringer's (a) or to Na-free Ringer's (b) on the serosal side. In both series, the mucosal bathing medium was Na-Ringer's. The data shown are from paired comparisons in four tissues. Values in Na-free serosal solution were obtained 10–20 min after Na^+ removal. V_{cs} and $a\text{Cl}_i$ in high- K^+ medium were measured 3 min after the substitution. Na^+ removal had no effect on $a\text{Cl}_i$. Moreover, the increases in $a\text{Cl}_i$ produced by raising serosal $[\text{K}]$ were not significantly different in the two experimental conditions. Means $\pm$ s.e.m. shown.

of Na^+ to this transport process on two grounds. First, removal of basolateral Na^+ did not cause a significant change of $a\text{Cl}_i$; and second, the increases of $a\text{Cl}_i$ produced by $[\text{K}]$ elevation were not different in Na^+ -free as compared to Na^+ -containing bathing medium. Na^+ removal caused a significant hyperpolarization of the basolateral membrane at the two serosal $[\text{K}]$ tested. This is in part caused by the paracellular diffusion potential produced by Na^+ replacement¹⁷. However, the depolarization produced by raising serosal $[\text{K}]$ was significantly larger in the absence of serosal Na^+ . This result could be explained by an increase of P_{K} secondary to elevated intracellular Ca^{2+} activity.

My finding of a KCl symport at the basolateral membrane of *Necturus* gallbladder is supported by recent studies of the steady-state effects of external $[\text{K}]$ on $a\text{Cl}_i$ ¹⁶ and of the mechanism of cell volume regulation after osmotic swelling²⁵; both studies were done in *Necturus* gallbladder. Na^+ -independent co-trans-

port of K^+ and Cl^- has also been demonstrated in red blood cells, both in response to osmotic swelling²⁶⁻²⁸ and after treatment with the sulphhydryl reagent *N*-ethylmaleimide^{29,30}. KCl co-transport has also been proposed to exist at the basolateral membrane of the thick ascending segment of the loop of Henle³¹. In *Necturus* gallbladder epithelium, the electrodiffusional Cl^- permeability of the basolateral membrane is very low¹⁰ and a Cl-HCO_3 (or Cl-OH) antiport would transport Cl^- into the cell, not out of it, as indicated by measurements of intracellular pH^9 . Therefore, KCl co-transport may well be the dominant basolateral Cl^- transport pathway in this and other NaCl -absorbing epithelia.

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Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon

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T-lymphocyte-mediated responses to the cellular components of blood vessels are important in rejection of allografts¹⁻³. The induction of cytolytic T lymphocytes (CTLs) depends on recognition of foreign class II major histocompatibility complex antigens (human HLA-DR, DC/DS, SB and others, collectively referred to as Ia) on the target cells whereas killing by CTLs usually depends on recognition of foreign class I antigens (HLA-A, B)⁴, although some alloreactive CTLs recognize foreign Ia instead of HLA-A, B (refs 5-8). The expression of Ia antigens has traditionally been regarded as restricted to immunological cell types, and the presence of class II antigen-bearing 'passenger' leukocytes in rodent organ grafts appears necessary for graft rejection⁹⁻¹¹. Recently, Ia antigens have been observed by immunofluorescence microscopy on human renal and dermal capillary endothelium¹²⁻¹⁵. We have previously shown that human umbilical vein endothelial (HUVE) cells in standard culture conditions do not bear Ia antigens, but may be induced to do so by products of lectin- or alloantigen-activated T lymphocytes^{16,17}. Furthermore, we found that recombinant immune interferon (IFN- γ), free of other lym-

phokines, is a potent inducer of Ia expression in HUVE cells¹⁷. Here we report that IFN- γ also induces Ia expression on human foreskin capillary endothelial (HFCE) cells, HUVE cells transformed by Simian virus 40 viral DNA (SV-HUVE cells) and human dermal fibroblast (HDF) cells in culture. Further, we present evidence that Ia present on HUVE cells and HDF cells can be functionally recognized by human T cells, resulting in a two-way interaction between T cells and mesenchymal cells that may be important in allograft rejection.

By monoclonal antibody binding and immune precipitation, HUVE¹⁸, SV-HUVE¹⁹, HFCE²⁰ and HDF²¹ cells in standard culture conditions express class I (HLA-A, B) but not class II (Ia) antigens (Table 1, Fig. 1). Fibroblast interferon (IFN- β) increases the level of expression of HLA-A, B in both HUVE and HDF, but does not induce Ia antigens (Table 1). In contrast, IFN- γ increases the level of expression of HLA-A, B and induces Ia antigens in all cells tested (Table 1, Fig. 1A). By fluorescence flow cytometry, IFN- γ uniformly converts the entire population of HUVE or HDF cells from Ia-negative to Ia-positive (not shown).

In primary HUVE cultures, it is possible that a non-endothelial contaminant blood cell synthesizes and sheds Ia which is subsequently adsorbed to the HUVE cell surface. As contaminating blood cells are not present in the multiply-passaged SV-HUVE cultures or the HFCE cultures used in these experiments, the demonstration that SV-HUVE and HFCE cells express (Table 1, Fig. 1A) and HFCE cultures biosynthesize (Fig. 1B) Ia antigens confirms that vascular endothelial cells synthesize Ia antigens. The response of the HDF strains suggests that this may be a property shared with other mesenchymal cell types. In addition to the alterations of the level of HLA-A, B and Ia, IFN- γ reproducibly induces expression of an unidentified polypeptide of molecular weight (M_r) ~17,000 in HUVE and HDF cells (Fig. 1A).

As the predominant proliferative response in the mixed leukocyte reaction is directed against Ia antigens²², we have attempted to use lymphocyte proliferation as an assay for T-cell recognition of endothelial cell and fibroblast Ia antigens. Primary HUVE cultures stimulate proliferation of allogeneic lymphocytes, whether or not Ia is expressed by the HUVE cells

Fig. 1 A, Composite autoradiogram comparing iodinated surface proteins of HUVE, HFCE and HDF-1 cells that had been either control-treated or IFN- γ -treated. The lanes are as follows: a, Total surface protein, control-treated HUVE cells; b, total surface protein, IFN- γ -treated HUVE cells; c-e, immune precipitations from control-treated HUVE cells using NRS, rabbit anti-human β_2 -microglobulin (R $\alpha\beta_2$) or rabbit anti-human Ia (R α Ia) xenosera respectively (both sera gifts of Dr Jack L. Strominger), f-h, immune precipitations from IFN- γ -treated HUVE using the same antisera; i-k, immune precipitations from control-treated HFCE using the same antisera; l-n, immune precipitations from IFN- γ -treated HFCE using the same antisera; o-q, immune precipitations from control-treated HDF-1 cells using the same antisera; r-t, immune precipitations from IFN- γ -treated HDF-1 cells using the same antisera; u, total surface protein, control-treated HDF-1 cells; v, total surface protein, IFN- γ -treated HDF-1 cells. B, Autoradiogram of gel with fluorographic enhancement (En³Hance, NEN) comparing immune-precipitated ³⁵S-methionine metabolically labelled proteins from HFCE cells analysed by SDS-polyacrylamide gel electrophoresis, as in A. Only the portion of the gel representing M_r below 50,000 is shown. Lanes a-c show precipitations from control-treated HFCE cells using NRS, R $\alpha\beta_2$ and R α Ia respectively, lanes d-f show precipitations from IFN- γ -treated cells using the same antisera. R $\alpha\beta_2$ precipitates HLA-A, B antigens (bands 1, 5) from both control- and IFN- γ -treated cells (lanes b, e) although the amount of material is larger in the IFN- γ -treated cultures. In contrast, R α Ia precipitates molecules only from IFN- γ -treated cells (doublet band 2, bands 3 and 4). Band 3 may correspond to the intracellular invariant chain of Ia antigens, detectable by metabolic but not surface labelling.

Methods: HUVE cells from several umbilical cords were pooled and grown in primary culture as described previously¹⁸. HFCE were isolated from a foreskin, grown from clonal density, and multiply passaged in the culture system described by Folkman *et al.*²⁰. HDF-1 cells, isolated from human foreskin, were donated by Dr James Rheinwald and multiply passaged in the same medium as HUVE cells except that 15% instead of 20% fetal bovine serum was used. IFN- γ was added as 0.2% medium conditioned by a transfected Chinese hamster ovary fibroblast line as described previously⁴³ (100 units interferon activity per ml assayed on FS4 cells), and replicate cultures were treated with control medium conditioned by a cell line transfected without the IFN- γ genes. In some lanes, immune precipitation from the total surface-iodinated proteins¹⁴ was used to identify class I or class II antigens, using R $\alpha\beta_2$ or R α Ia. The specificity of precipitation is shown by comparison with normal rabbit serum (NRS). SDS-polyacrylamide gel electrophoresis was performed using the buffer system of Laemmli on 20-cm long 7-15% acrylamide gradients.

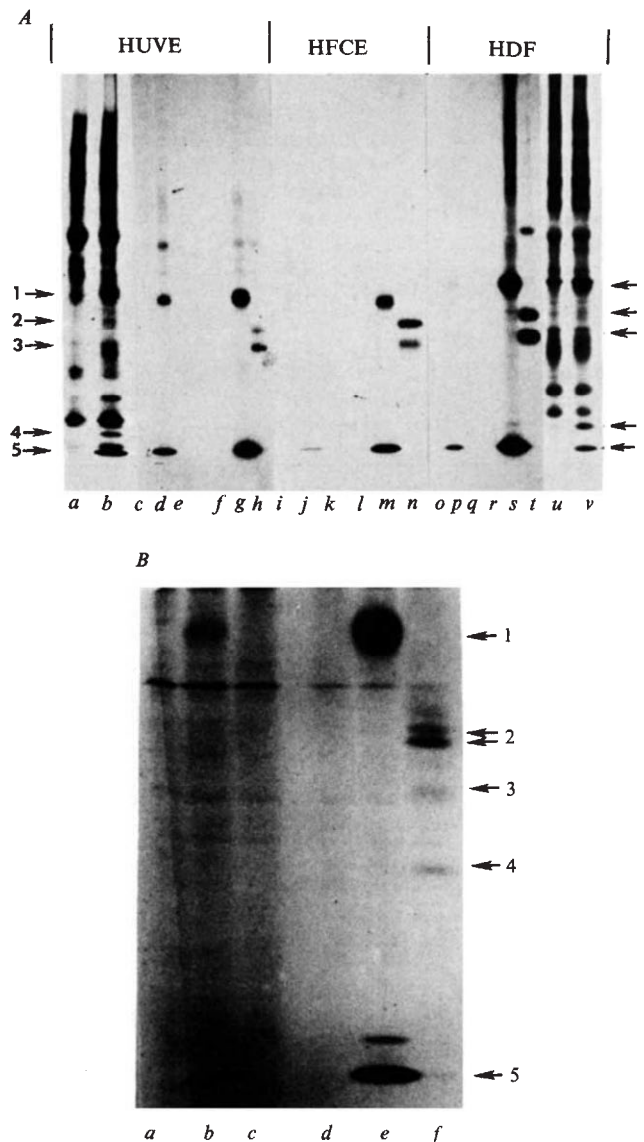


Table 1 Effects of IFN- β and IFN- γ on MHC antigens

Cell type	HLA-A, B			HLA-DR			Background		
	Control	IFN- β	IFN- γ	Control	IFN- β	IFN- γ	Control	IFN- β	IFN- γ
Expt 1		W6/32 binding			I2 binding			UPC10 binding	
HUVE	13.6 $\pm$ 1.4	23.1 $\pm$ 1.5	26.4 $\pm$ 2.1	1.1 $\pm$ 0.1	1.5 $\pm$ 0.3	9.6 $\pm$ 0.4	1.4 $\pm$ 0.02	2.2 $\pm$ 0.5	1.9 $\pm$ 0.2
HDF-1	19.9 $\pm$ 1.2	30.8 $\pm$ 1.8	31.6 $\pm$ 1.1	2.2 $\pm$ 0.1	3.1 $\pm$ 1.9	6.9 $\pm$ 0.5	3.5 $\pm$ 1.2	2.2 $\pm$ 0.3	2.5 $\pm$ 0.3
HDF-2	20.8 $\pm$ 1.0	29.6 $\pm$ 0.4	29.4 $\pm$ 2.5	1.8 $\pm$ 0.1	2.0 $\pm$ 0.4	8.6 $\pm$ 0.7	1.9 $\pm$ 0.3	2.0 $\pm$ 0.3	2.5 $\pm$ 0.1
HDF-3	13.4 $\pm$ 1.2	27.0 $\pm$ 2.1	31.9 $\pm$ 1.9	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	7.1 $\pm$ 0.2	1.4 $\pm$ 0.2	1.7 $\pm$ 0.6	2.2 $\pm$ 0.3
HDF-4	13.1 $\pm$ 0.8	24.6 $\pm$ 1.5	27.5 $\pm$ 1.3	1.0 $\pm$ 0.02	1.1 $\pm$ 0.1	8.0 $\pm$ 0.6	0.8 $\pm$ 0.03	1.2 $\pm$ 0.6	1.2 $\pm$ 0.01
HDF-5	14.7 $\pm$ 0.6	25.8 $\pm$ 0.8	24.6 $\pm$ 2.4	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	6.6 $\pm$ 0.1	1.3 $\pm$ 0.7	1.7 $\pm$ 0.9	1.3 $\pm$ 0.1
Expt 2		W6/32 binding			LB3.1 binding			UPC10 binding	
HFCE	29.8 $\pm$ 0.7	ND	67.5 $\pm$ 15.5	4.1 $\pm$ 0.1	ND	50.6 $\pm$ 0.7	4.9 $\pm$ 0.4	ND	5.2 $\pm$ 0.2
Expt 3		BBM.1 binding			LB3.1 binding			UPC10 binding	
HUVE	21.4 $\pm$ 2.2	ND	30.4 $\pm$ 2.0	1.6 $\pm$ 0.04	ND	14.7 $\pm$ 2.4	2.0 $\pm$ 0.3	ND	4.2 $\pm$ 4.6
HFCE	18.4 $\pm$ 2.8	ND	36.6 $\pm$ 3.9	3.6 $\pm$ 1.6	ND	31.5 $\pm$ 0.8	1.8 $\pm$ 0.1	ND	2.1 $\pm$ 0.3
Expt 4		BBM.1 binding			LB3.1 binding			UPC10 binding	
HUVE	49.6 $\pm$ 3.8	ND	87.4 $\pm$ 1.3	4.3 $\pm$ 2.3	ND	55.3 $\pm$ 3.7	2.2 $\pm$ 0.2	ND	2.4 $\pm$ 0.7
SV-HUVE	68.0 $\pm$ 13.0	ND	83.8 $\pm$ 7.6	5.3 $\pm$ 1.3	ND	16.1 $\pm$ 1.8	5.0 $\pm$ 1.1	ND	3.6 $\pm$ 0.9

Expression of class I and class II histocompatibility antigens by HUVE, SV-HUVE, HFCE and HDF cultures measured by monoclonal antibody binding quantitated¹⁶ by second antibody binding using ¹²⁵I-sheep anti-mouse immunoglobulin, F(ab')₂ fragment (NEN). The specific activity of the second antibody differs among these experiments. Class I antigens were measured with mouse monoclonal antibodies BBM.1 (anti-human β_2 -microglobulin) and W6/32 (anti-human HLA-A, B framework, gifts of Dr Peter Parham). Class II antigens were measured with LB3.1 and I2 (anti-human HLA-DR framework, gifts of Drs Peter Knudsen and Lee Nadler). Nonspecific binding was measured using UPC-10, a mouse myeloma protein of IgG2a subclass (Walgene R & D Laboratories). Cells were compared when cultured in standard conditions or supplemented with IFN- β (produced from a cloned human gene in *Escherichia coli* and purified to homogeneity, units not assayed, a gift of Biogen) or IFN- γ (produced from a cloned human gene in Chinese hamster ovary fibroblasts⁴², added as conditioned medium diluted to a final concentration of 0.2% or 100 units ml⁻¹) for 3 days as indicated. Values are c.p.m. $\times 10^{-3}$ per well $\pm$ s.d. ND, not done.

Table 2 Specific lysis by cloned human CTL line CF-1

	HUVE 1		Target		HUVE 2		HDF-5	
	¹¹¹ In release	Specific lysis	¹¹¹ In release	Specific lysis	¹¹¹ In release	Specific lysis	¹¹¹ In release	Specific lysis
Control pretreated								
NP40	152.9 ± 5.6		98.9 ± 10.0				100.2 ± 7.3	
Spontaneous	9.4 ± 1.1		36.6 ± 3.3				9.8 ± 1.2	
LB3.1 + C'	11.8 ± 0.3	1.6%	41.3 ± 2.1	7.6%			9.6 ± 2.0	—
CF-1	12.5 ± 2.2	2.1%	41.8 ± 2.3	8.4%			27.1 ± 4.2	19.2%
CF-1 + Anti-DR	12.2 ± 1.8	1.9%	31.7 ± 1.6	—			20.6 ± 1.9	22.9%
IFN-γ pretreated								
NP40	141.0 ± 7.2		114.9 ± 22.9				115.8 ± 16.1	
Spontaneous	9.0 ± 0.2		33.8 ± 2.0				8.2 ± 0.2	
LB3.1 + C'	75.0 ± 1.0	50.0%	85.8 ± 18.4	64.2%			62.0 ± 6.7	50.0%
CF-1	41.3 ± 5.3	24.4%	61.3 ± 3.8	33.9%			54.6 ± 3.1	43.1%
CF-1 + Anti-DR	13.8 ± 1.1	3.5%	32.7 ± 1.5	—			19.0 ± 2.7	10.1%

Cells in monolayer cultures in Costar 96-well microtitre plates were control treated or treated with IFN-γ (100 units ml⁻¹) for 3 days before assay as in Table 1. Microtitre wells were washed twice with complete Hank's balanced salt solution (HBSS), labelled with 2 μCi per well of indium-111 oxine (Amersham) in 0.20 ml HBSS for 15 min at 22 °C, then washed once with Tyrode's solution containing 40 mg ml⁻¹ bovine serum albumin, 1 mg ml⁻¹ glucose, pH 7.4 (Tyrode's-albumin). The samples were incubated for 90 min at 37 °C and then washed once more with the Tyrode's-albumin and once with minimal Eagle's medium (MEM) containing 10% fetal calf serum (M.A. Bioproducts, SMEM-10). 2.0–3.0 × 10⁵ CF-1 cells (effector:target of 10–15:1) were added to some wells in SMEM-10, and some wells with CF-1 cells were additionally supplemented with TS1 16.1.1 (an IgG mouse monoclonal antibody against HLA-DR) at 1:10 final dilution of culture supernatant. SMEM-10 was added to all wells to make a total assay volume of 0.2 ml. After 5 h at 37 °C, 0.1 ml of assay supernatant was collected from appropriate wells (without lymphoid cells) which were then washed once with SMEM-10 and treated with either 2.0% Nonidet P-40 (NP40, Sigma, detergent lysis) or LB3.1 (an IgG2b mouse monoclonal antibody against HLA-DR) at a final concentration of 1:50 ascites fluid by volume. After 30 min at 37 °C, the LB3.1 monoclonal antibody was removed by washing twice with SMEM-10, and baby rabbit complement (C', Pel-Freeze) diluted 1:1 with SMEM-10 was added. 30 min later, at 6 h of total incubation at 37 °C, the microtitre plates were centrifuged briefly, 0.1 ml of supernatant was removed, and released ¹¹¹In was measured using a Beckman model Gamma 8000. Supernatants from the wells treated with NP40 or LB3.1 plus complement were pooled with the 0.1 ml removed at 5 h so as to include ¹¹¹In released spontaneously during the first 5 h. Data are shown both as means of triplicates ± s.d. and as per cent specific release of radioactivity. Per cent specific release was calculated as follows: (c.p.m. experimental – c.p.m. spontaneous)/(c.p.m. total (2% NP40) – c.p.m. spontaneous). The data are from three separate experiments (each HUVE culture is the one of five single cord cultures for which CF-1 killing was seen; HDF-5 is the only one of five dermal fibroblast strains susceptible to CF-1 killing). IFN-γ pretreated HUVE-1 showed specific lysis of 63.9% with an alloserum against HLA-DR6; three other single cord cultures not susceptible to CF-1 killing were unaffected by alloserum and one culture could be killed by alloserum but not by CF-1. ¹¹¹In release values given are c.p.m. × 10⁻³ per well ± s.d.

before the onset of co-culture with lymphocytes (Fig. 2). The ability of initially Ia-negative HUVE cells to stimulate proliferation may be explained by our previous observation that during co-culture with allogeneic lymphocytes, HUVE cells rapidly become Ia positive¹⁷. The induction of HUVE Ia antigens may be triggered through activation of a few T cells by Ia-positive leukocytes contaminating the HUVE primary cultures, leading to IFN-γ production, or perhaps through presentation of HUVE class I antigens by monocytes in the blood lymphocyte population²³. Our preliminary experiments have shown that SV-HUVE also stimulate lymphocyte proliferation, supporting the interpretation that endothelial cells, rather than contaminating leukocytes, are predominantly responsible for the proliferative lymphocyte response. Fibroblasts, whether Ia positive or Ia negative at the start of co-culture, stimulate much less proliferation than endothelial cells; in fact, these same fibroblast cultures seem actively to inhibit lymphocyte proliferation. The inhibition cannot be overcome by exogenous interleukin-1 (a gift of Dr Charles Dinarello) nor by inhibitors of prostaglandin synthesis, nor can it be transferred in conditioned medium (not shown). The physiological significance of this *in vitro* observation is unknown, but it limits the use of T-cell proliferation to assess recognition of fibroblast Ia.

A cloned human CTL line (CF-1) specific for an HLA-DR antigen (HLA-DR6 specific, produced as in ref. 24 and described in detail elsewhere²⁵), was used to determine whether T cells could recognize Ia induced by IFN-γ on the surface of HUVE and HDF cells. CF-1 effectively kills both HUVE and HDF cells, as judged by an ¹¹¹In release assay²⁶ (Table 2). In each case, CF-1 selectively kills cells pretreated with IFN-γ so as to express Ia, and the degree of killing by CF-1 can be significantly inhibited by a monoclonal antibody specific for an HLA-DR framework determinant. None of eight other parallel single donor HUVE cultures or of four other HDF strains can be killed by CF-1, with or without pretreatment by IFN-γ (not

shown), suggesting that only specific DR alloantigens are being recognized in the killing assay. Morphologically, those wells which indicate killing by indium release reveal extensive rounding up of the target cells, confirming the occurrence of target cell injury.

IFN-γ has antiviral interferon-like actions, shared with leukocyte (IFN-α) and fibroblast (IFN-β) interferons, and additional immune regulatory properties²⁷. Among the latter, IFN-γ has been shown to induce the expression of class II (Ia) antigens on mouse macrophages²⁸, human monocytes^{29,30}, mouse mast cells³¹, human melanoma cells³⁰ and human vascular endothelial cells¹⁷ *in vitro*. (Human B lymphoblastoid cells, which are already Ia-positive, do not appear to respond further to IFN-γ.)³² *In vivo*, an activated T-cell product(s), possibly IFN-γ, recruits Ia-positive monocytes from mouse bone marrow³³ and induces both rodent and human skin and intestinal epithelial cells to express Ia^{34–37}. We report here that three different types of cultured human endothelial cells can be induced to express Ia *in vitro* by IFN-γ. This response of endothelial cells is not limited to endothelium because five different strains of human dermal fibroblast cells also make Ia in response to IFN-γ. In addition to Ia induction, IFN-γ increases levels of class I antigens and induces the expression of an unidentified 17,000-M_r surface antigen. It has recently been shown that IFN-γ induces several major polypeptides (not similarly affected by IFN-α or -β) in human fetal lung fibroblasts, and some of these have appropriate molecular weights for class I and II antigens although they were not identified as such³⁸.

Our experiments with the cloned human CTL line establish that T cells can recognize the Ia molecule on the surface of both endothelium and fibroblasts. The CF-1 clone has been extensively characterized. It is OKT3⁺, OKT4⁺, OKT8[–] and killing of lymphoblastoid targets can be blocked by monoclonal antibodies against HLA-DR but not against HLA-A, B or DC antigens²⁵. Furthermore, its target specificity correlates well

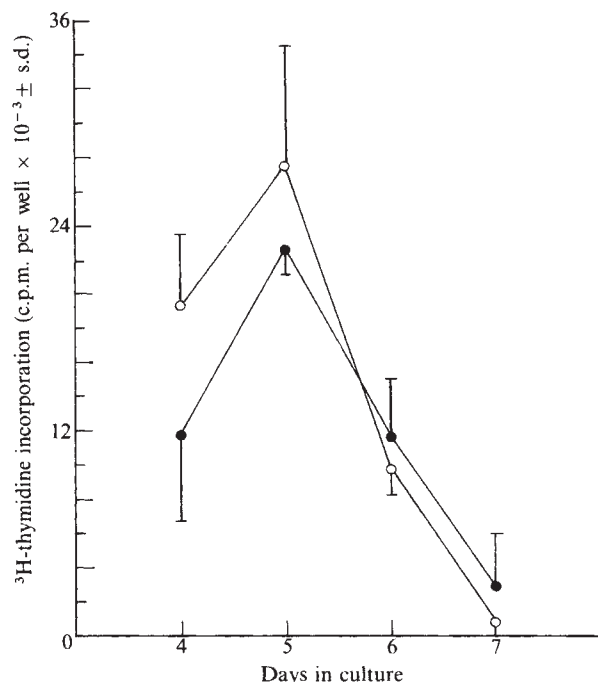


Fig. 2 Time course of proliferation of peripheral blood mononuclear cells stimulated by allogeneic HUVE cultures, with (○) or without (●) pretreatment of the HUVE cultures with IFN- γ . Proliferation is measured by incorporation of ^3H -thymidine into macromolecules as described previously¹⁵, using 3×10^5 responder cells and a γ -irradiated (1,200 rad) confluent Costar 96-microtitre well (M.A. Bioproducts) of HUVE cells as stimulators ($\sim 1.5 \times 10^4$ cells). Incorporation is corrected for incorporation of the responder cells and stimulator cells cultured separately. In parallel experiments, γ -irradiated monolayers of HDF-1 and HDF-5 cells stimulate little or no significant incorporation of ^3H -thymidine into lymphocytes from any of three different blood donors (each of which cross-stimulates the other two). The lack of stimulation is seen even after IFN- γ pretreatment of the HDF cells so as to express comparable amounts of Ia antigens per microtitre well to HUVE cells.

with the serologically determined HLA-DR6 antigen, as deduced from a panel of lymphoblastoid targets. In the data presented here, only IFN- γ -pretreated HUVE and HDF cells (that is, Ia-positive cells) are specific targets for killing, and cytotoxicity can be blocked by mouse anti-human DR monoclonal antibody. Other mouse monoclonal antibodies, such as W6/32 directed against HLA-A, B antigens, are not inhibitory (not shown). Only 3 cultures in 15 were suitable targets, the expected frequency of the CF-1 target determinant (HLA-DR6), although it was not possible to tissue type the donors of these cells. In the one experiment tested, we found a correlation between susceptibility to killing of HUVE cells by CF-1 and by anti-HLA-DR6 alloserum.

The killing of endothelial targets in the HUVE cultures cannot be attributed to the presence of a small contaminating cell subpopulation, because CTLs must recognize and bind to the target molecule on the surface of the cell undergoing lysis (that is, CTLs do not damage bystander cells). Thus, if a small subpopulation of contaminating cells were the only cells bearing functional Ia, only those cells should be killed. In fact, most cells show morphological evidence of damage and the amount of radioactive indium release confirms membrane injury to a significant percentage of cells.

Our results have several implications. The demonstration that endothelial cells synthesize and express 'functional' foreign Ia (demonstrated by CTL recognition) indirectly supports previous conclusions that human endothelial cells can present foreign antigen to sensitized T cells in combination with functional

self-Ia^{39,40}. Second, the demonstration that Ia antigens on human mesenchymal cells can be recognized by alloreactive T cells suggests that the elimination of Ia-positive passenger leukocytes from human organ grafts may not by itself be sufficient to extend graft survival; Ia biosynthesis by vascular and other mesenchymal cell components of the allograft, which is subject to induction by T cells, may also need to be suppressed. Third, the existence of class II-specific CTLs of OKT4-positive phenotype, like CF-1, that can kill vascular cells, may limit the clinical usefulness of OKT4/OKT8 ratios in assessing the 'immunological state' of a response to an organ graft^{41,42}. Interestingly, the Ia-specific cloned CTL line used in these experiments secretes IFN- γ and medium conditioned by this cell line induces endothelial Ia (J.S.P., unpublished data). Thus, endothelial cells and T cells conduct a dialogue that may be important for allograft rejection.

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Cessation of cytokeratin expression in a rat hepatoma cell line lacking differentiated functions

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Intermediate-sized filaments (IF) of diameter 7–11 nm occur in the cytoplasm of most cells of vertebrates and their constituent proteins are abundant in most cell types. Expression of IF proteins depends on the route of cell differentiation and five major subclasses of IF proteins have been distinguished^{1–5}: of these, cytokeratins are typical of epithelial cells whereas vimentin occurs in mesenchymally derived cells and some other non-epithelial cells. When epithelial cells are grown in culture this restriction of IF expression is often lost and they begin to synthesize vimentin in addition to cytokeratin^{5–9}, although examples of maintenance of the cell-type-specific expression of only cytokeratin have also been reported^{8,10}. No IFs have been detected in mammalian germ cells or in pre-morula stages of mouse embryogenesis, and the first IF proteins identified in murine blastocysts are cytokeratins of trophectodermal cells^{11,12}. We report here that a dedifferentiated¹³ rat hepatoma cell clone, which has become resistant to the action of the glucocorticoid hormone analogue dexamethasone and has lost various liver-specific functions, also stops all synthesis of IF proteins, without obvious consequences for growth and proliferation. The existence of such cells devoid of IF supports the notion that such filaments are not involved in basic cellular functions necessary for growth and proliferation but are related to special functions of the differentiated cell.

Faza 967 cells are cultured cells of an 8-azaguanidine-resistant clonal line derived from carcinogen-induced Reuber H35 rat hepatoma¹⁴. The growth of these cells is inhibited by dexamethasone¹⁵ and they express several liver-specific functions, including basal and glucocorticoid-inducible tyrosine aminotransferase (TAT), gluconeogenic enzymes, liver-type isoenzyme patterns of alcohol dehydrogenase and aldolase and secretion of serum albumin ('differentiated Faza 967 cells')¹⁴. Faza 967 cells that have been grown for more than 3 yr in culture have reduced levels of liver-specific functions ('partially dedifferentiated Faza 967 cells')¹⁶. From differentiated Faza 967 cells dexamethasone-resistant clonal sublines have been isolated, some of which (for example Dex-Faza 967 clone D2) are also characterized by reduced expression of certain liver-specific functions such as TAT^{15,17}. Cells of clone D2 grown for 8 months on dexamethasone-containing medium and then for more than 3 yr without additions of the hormone analogue (Dex-Faza 967 clone 2) appear to be dedifferentiated in that they no longer exhibit the liver-specific functions examined (Table 1 and ref. 17).

When examined by immunofluorescence microscopy the original differentiated Faza 967 cells as well as the partially dedifferentiated Faza 967 and Dex-Faza 967 clone D2 cells all contain IF exclusively of the cytokeratin type (Fig. 1a–c), similar to hepatocytes of rat liver tissue and various other rat hepatoma cell lines^{8,18}. By contrast, cells of another Reuber hepatoma-derived cell line, H56, which are also sensitive to dexamethasone but are dedifferentiated (Table 1; refs 14, 15, 19), do not reveal fibrillar staining with antibodies to cytokeratins (Fig. 1d). Instead H56 cells are rich in vimentin IF (Fig. 1e) which do not occur in hepatocytes of liver tissue but frequently appear *de*

novo in hepatocytes and hepatoma cells growing in culture^{8,20}. However, dexamethasone-resistant dedifferentiated clone 2 cells are negative for both vimentin and cytokeratin (Fig. 1f–h). Desmin, an IF protein typical of myogenic cells^{1–4}, and desmoplakins, major constituent proteins of desmosomes^{21,22}, have not been detected by immunofluorescence microscopy in any of the cell lines listed in Table 1. Correspondingly, electron microscopy of whole cells and cytoskeletal residues has shown that Faza 967 cells as well as clone D2 cells are devoid of desmosomes but contain IF, sometimes arranged in fasciated bundles characteristic of cytokeratin IF arrays of many epithelial cells (Fig. 1j,l). H56 cells also lack desmosomes but have IF which are organized into loose bundles (Fig. 1k) typical of vimentin. By contrast, we have not detected any IF in intact clone 2 cells and cytoskeletons made therefrom. The only structures identified in such high-salt-extracted cytoskeletal preparations are residual nuclear structures, including fragments of the nuclear lamina (not shown).

Cytoskeletons of rat hepatocytes contain two cytokeratin polypeptides designated A (apparent molecular weight (M_r) 55,000; two major isoelectric variants of apparent pI 6.40 and 6.45) and D (M_r 49,000; pI of major variant ~5.4)^{18,23}, corresponding to cytokeratins 8 and 18 of the human cytokeratin catalogue²⁴. These two hepatic cytokeratins are also found on two-dimensional gel electrophoresis of cytoskeletal proteins from differentiated and partially dedifferentiated Faza 967 cells (Fig. 2a) and from Dex-Faza clone D2 cells (Fig. 2b). In all these cells, cytokeratins A and D are the only IF proteins identified. By contrast, in analyses of cytoskeletons from H56 cells we have recognized only vimentin (Fig. 2c). However, cytoskeletons from cells of Dex-Faza 967 clone 2 have revealed neither cytokeratins nor vimentin (Fig. 2d). In these cells the only major cytoskeletal protein remaining after extraction with high-salt buffer and Triton X-100 is an acidic polypeptide of M_r 66,000 which is present in most vertebrate cells (designated Z in Fig. 2a–f and ref. 11) and has recently been identified by peptide mapping as nuclear lamin B (not shown). Figure 2e presents a separation of cytoskeletal proteins from clone 2 cells by non-equilibrium pH-gradient gel electrophoresis, demonstrating the absence of basic cytokeratins²⁵. Absence of cytokeratins and vimentin in clone 2 cells has also been confirmed by sensitive silver staining²⁶ (not shown) and by autoradiofluorography using proteins labelled with ³⁵S-methionine *in vivo* (Fig. 2f).

We have recently introduced, as a very sensitive technique to detect small amounts of soluble IF proteins, co-polymerization by addition of an excess of homologous or heterologous IF proteins of the same type^{27,28}. The high resolution of this procedure is exemplified in Fig. 3a, b which shows the specific enrichment of ³⁵S-methionine-labelled cytokeratin polypeptides A, D and M_r 40,000 from *in vitro* translation assays using RNA isolated from Novikoff rat hepatoma cells after IF reconstitution with added unlabelled bovine epidermal keratins. No such polypeptides can be detected when bovine epidermal keratins are incubated together with ³⁵S-methionine-labelled clone 2 cell proteins of supernatants obtained after 10 min centrifugation at 12,000g (Fig. 3c, d), indicating the absence of soluble IF proteins in these cells. Likewise, no IF proteins have been recovered in pelletable form when Triton X-100 and high-salt buffer extracts from ³⁵S-methionine-labelled clone 2 cells have been dialysed against 10 mM Tris-HCl buffer (pH 7.4) and mixed with unlabelled epidermal keratins in IF reconstitution conditions^{28,29} (not shown). To examine whether IF proteins are rapidly degraded by proteases unusually active in clone 2 cells we have mixed ³⁵S-methionine-labelled Faza 967 cells with the threefold excess of unlabelled clone 2 cells and prepared cytoskeletal proteins from this mixture. On two-dimensional gel electrophoresis we have recognized cytokeratins A and D in amounts comparable to those made from Faza 967 cells alone. Finally, when poly(A)⁺ RNA prepared from clone 2 cells was examined by *in vitro* translation (for preparation, see refs 27, 28) neither cytokeratin nor vimentin was found (not shown).

Fig. 1 Immunofluorescence (*a-h*) and electron (*j-l*) microscopy of cultured rat hepatoma cells. Culture conditions^{15,17}, antibodies to cytokeratins and vimentin^{18,25,38,39} and procedures used for microscopy^{10,20,27,29,39} have been described previously. Scale bars: *a-h*, 20 μ m; *j-l*, 0.2 μ m. *a*, Differentiated Faza 967 cells stained with anti-cytokeratin antibodies. *b*, Same cells as in *a* but stained with anti-vimentin antibodies. *c*, Partially dedifferentiated Dex-Faza 967 clone D2 cells stained with anti-cytokeratin antibodies. *d*, Dexamethasone-sensitive rat hepatoma cells of H56 line after reaction with anti-cytokeratin antibodies. *e*, Same H56 cells but stained with anti-vimentin antibodies. *f*, Dexamethasone-resistant dedifferentiated Faza 967-derived clone 2 cells after reaction with anti-vimentin antibodies. *g*, Phase-contrast picture of cells shown in *f*. *h*, Clone 2 cells after reaction with anti-cytokeratin antibodies. *i*, Phase-contrast picture of *h*. *j*, Electron microscopic section through cortical portion of intact cell of Dex-Faza 967 clone D2 cell, showing typical densely fasciated bundles of cytokeratin filaments (arrows). *k*, Electron microscopy of cell of H56 line showing loosely arranged bundle of vimentin IF. *l*, Electron microscopy of pellet of cytoskeletal material from Dex-Faza 967 clone D2 cells isolated by extraction in buffers containing 1.5 M KCl and 1% Triton X-100 as described previously^{8,25}, showing typical IF with hollow cores (insert).

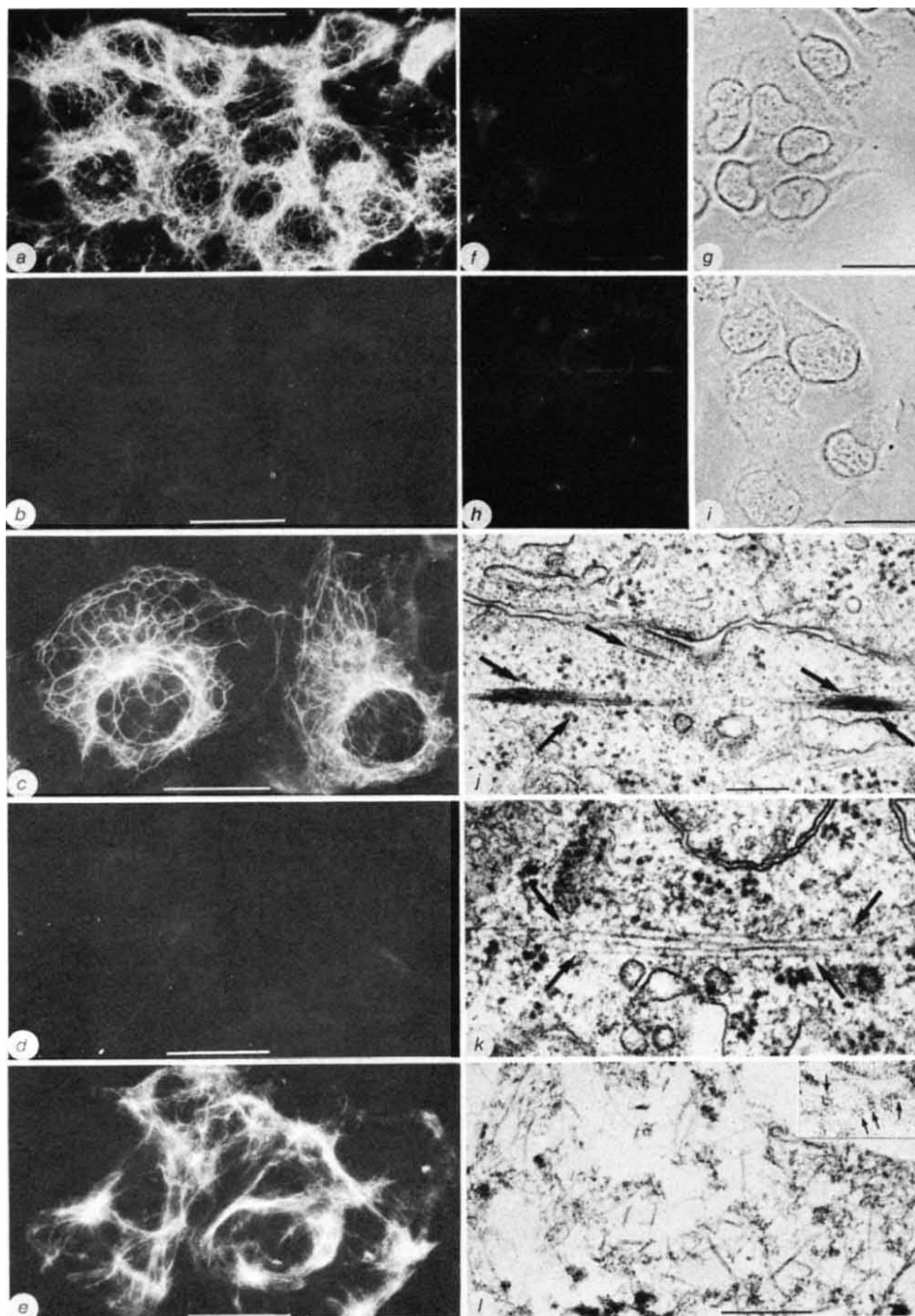


Table 1 Liver-specific functions and cytoskeletal proteins of some Reuber hepatoma-derived rat clonal cell lines

Rat hepatoma cells	Ref.	TAT activity		L-ADH	Ald B	Serum albumin (μ g secreted per 10 ⁶ cells per 48 h)	Desmoplakins*	Cytokeratins*	Vimentin*
		Basal	Induced						
Faza 967	16	50	330	+	+	3.3	—	+	—
Partially dedifferentiated									
Faza 967	16	7	10	+	+	0.2	—	+	—
Clone D2	17	5	24	—	+	4.3	—	+	—
Clone 2	17	8	10	—	—	<0.1	—	—	—
H56	14, 15	1	1	—	—	—	—	—	+

Partially dedifferentiated Faza 967 cells had been grown for more than 3 yr in culture (see text). Induction of TAT was performed as described in ref. 15. The presence (+) of liver-specific alcohol dehydrogenase (L-ADH) and aldolase (Ald B) was detected by electrophoretic analysis of cell extracts (for details see refs 16, 17).

* Detected by immunofluorescence microscopy and gel electrophoresis.

Fig. 2 Two-dimensional gel electrophoresis (IEF, direction of isoelectric focusing; NEPHGE, direction of non-equilibrium pH-gradient electrophoresis; downward arrows indicate second-dimension electrophoresis in the presence of SDS; for details see refs 17, 21, 25) of cytoskeletal polypeptides from various rat Reuber hepatoma-derived cell lines. Cytoskeletons were prepared as described in ref. 8, except that centrifugation was for 20 min at 12,000g instead of 3,500g. Gels *a-e* are stained with Coomassie blue, *f* is an autoradiograph. Reference proteins used for co-electrophoresis were bovine serum albumin (BSA), α -actin (α) and phosphoglycerokinase (PGK); residual β - and γ -actin are also indicated; cyokeratin polypeptides are denoted A and D, vimentin V (small arrows denote degradation products of vimentin), and the polypeptide of M_r 66,000 is designated Z. *a*, Differentiated Faza 967 cells; *b*, partially dedifferentiated Dex-Faza 967 clone D2; *c*, dedifferentiated cells of line H56; *d*, dedifferentiated Dex-Faza 967 clone 2; *e*, same cytoskeletal preparation, showing absence of basic cyokeratins (isoelectric point of PGK = pH 7.5); *f*, cytoskeletal polypeptides of same cell clone 2 visualized by fluororadiography (long exposure) of proteins labelled with 35 S-methionine *in vivo*⁸. None of the minor radioactive spots coincides with rat cyokeratins A and D or vimentin co-electrophoresed and visualized by Coomassie blue staining.

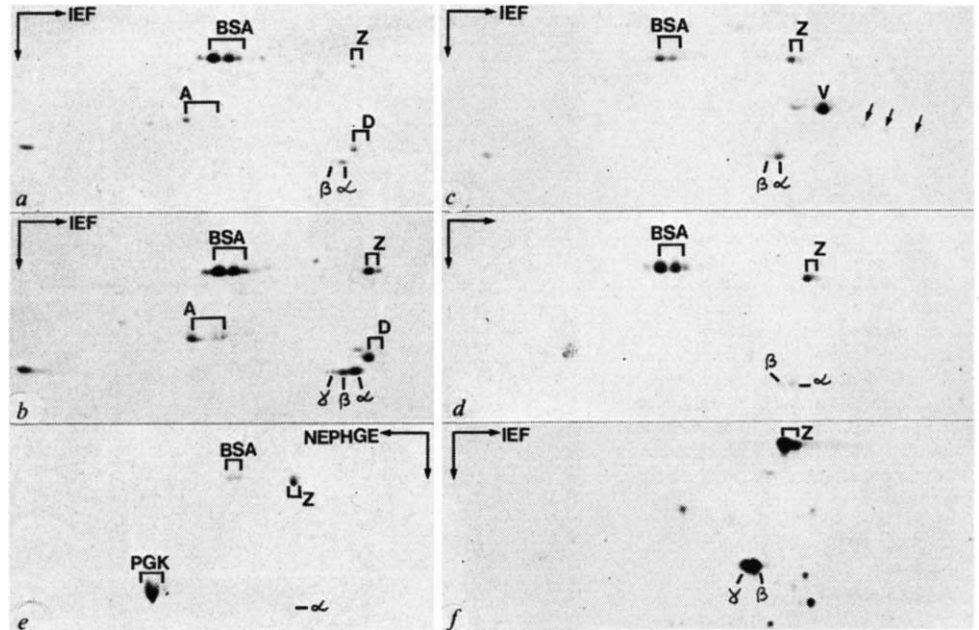
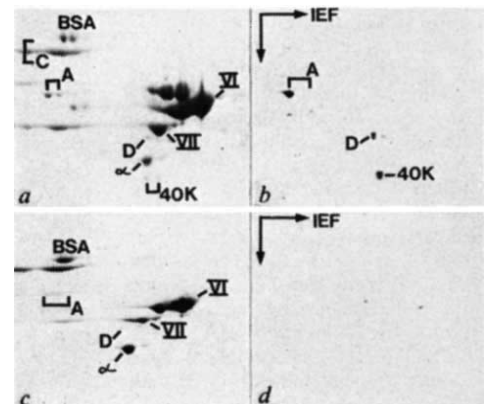


Fig. 3 Two-dimensional gel electrophoresis of cytoskeletal proteins of hepatoma cell proteins, showing specific enrichment of soluble cyokeratins by co-polymerization with added homologous and heterologous cyokeratins. *a*, *b*, 35 S-methionine-labelled translational products from poly(A)⁺ RNA of Novikoff rat hepatoma cells, using the reticulocyte lysate system, were mixed with an excess of cyokeratins from bovine muzzle epidermis and allowed to polymerize^{27,28}. Then cytoskeletal material from Novikoff hepatoma cells was added and the mixture was extracted by high-salt buffer and Triton X-100 (refs 27, 28). The insoluble residue containing IF was analysed by gel electrophoresis. *a*, Coomassie blue staining, showing major bovine epidermal keratin polypeptides (Roman numerals according to refs 18, 25, 27), Novikoff hepatoma cyokeratins (A, D and 40K) and co-electrophoresed bovine serum albumin (BSA) and α -actin (α). *b*, Fluororadiograph of the same gel, showing the specific enrichment of three cyokeratins (A, D, 40K)^{39,40} from total soluble translation products of Novikoff rat hepatoma mRNA by co-polymerization with the unlabelled cyokeratin added (*a*). *c*, *d*, Approximately 2×10^7 dexamethasone-resistant clone 2 cells were labelled with 35 S-methionine for 12 h as described previously⁸, homogenized in 10 mM NaCl, 1% Triton X-100, 10 mM EGTA, 10 mM EDTA, 0.4 mM phenylmethylsulphonyl fluoride, 10 mM Tris-HCl (pH 7.4). Soluble proteins were obtained as supernatants after 10 min centrifugation at 12,000g. To this supernatant (~ 0.1 mg protein ml⁻¹) an excess of total bovine epidermal cyokeratins was added in the same buffer as used in *a*. After 30 min incubation reconstituted IF material was treated with high-salt buffer⁸, pelleted, washed with 10 mM Tris-buffer (pH 7.2) containing 1 mM EDTA and analysed by gel electrophoresis. *c*, Coomassie blue staining (labelling as in *a* and Fig. 2). *d*, Fluororadiograph showing absence of significant amounts of radioactive cyokeratins in the pellet. Exposure was for such a long time that minor background spots can be detected.



The fact that hepatoma cells of the clone 2 grow and proliferate with a generation time (19.3 h) similar to that of their parental differentiated Faza 967 cells³⁰ demonstrates that neither liver-specific functions nor IF are necessary for such basic cellular events as growth and proliferation, cell coupling and colony formation. These findings also show that IF are not obligatory structures required for anchorage of the cell nucleus, as suggested by some authors^{1,31}. The conclusion that, in cultured cells, IF are not involved in fundamental functions is also consistent with results of microinjection of antibodies to cyokeratins or vimentin³²⁻³⁵ and the reported absence of IF in pre-morula stages of mouse embryos^{11,12}. Cells of clone 2 are morphologically different from Faza 967 cells in that they are relatively flat and do not form trabecular colonies. However, whether this morphological change is related to the change in IF expression is not clear. Our finding of an absence—or extremely low levels (level of detection of cyokeratins with the methods used $\sim 3 \times 10^4$ molecules per cell)—of IF proteins in hepatoma cells of this line provides the first example of a cessation of IF expression during culturing and the maintenance

of this IF-deficient state in a permanent cell line. Comparison of the different derivatives from the original Faza 967 cell clone shows that dexamethasone resistance is not a prerequisite for the loss of expression of IF as the Dex-Faza 967 clone D2 produces considerable amounts of the cyokeratins which are present in the glucocorticoid-sensitive parental cells. Cyokeratin is present in partially dedifferentiated Faza 967 and vimentin occurs in the dedifferentiated rat hepatoma cell lines H56 and HTC⁸. Thus loss of certain liver-specific function *in vitro* does not necessarily correlate with loss of cyokeratins or *de novo* expression of vimentin. Independent loss and re-expression of differentiation markers has also been observed for other proteins of cultured hepatocytes and hepatoma cells^{14,36,37}.

Clearly, the sequence of disappearance of differentiation markers, including IF proteins, during culturing needs further study. Among 17 independently established Reuber hepatoma-derived clonal cell lines examined, 7 have maintained cyokeratin IF expression without producing vimentin, 7 synthesize vimentin but not cyokeratin, 2 produce simultaneously

both cytokeratin and vimentin, and 1 (clone 2) has lost the expression of both types of IF protein. This and previous observations in other cells lines⁵⁻¹⁰ suggest that complete loss of IF during *in vitro* culture may not be a frequent happening.

It will also be important to find out whether the loss of IF expression *in vitro* is irreversible. Preliminary observations of occasional cytokeratin-positive cells in clone 2 cultures (11 out of ~14,000 cells examined by immunofluorescence microscopy)

have prompted us to look for reversions of IF expression and for culture conditions favouring re-expression.

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Association of human T-cell leukaemia/lymphoma virus with the Tac antigen marker for the human T-cell growth factor receptor

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Certain adult T-cell lymphoproliferative disorders are associated with human T-cell leukaemia virus (HTLV), a unique human type C retrovirus^{1,2}. (The strains of HTLV used in these studies belong to the subgroup HTLV-I.) HTLV is not an endogenous agent in man, but rather is an acquired virus with T-cell tropism^{3,4}. Neoplastic cells from patients infected with HTLV generally express receptors for T-cell growth factor (TCGF) (interleukin-2)^{5,6}, and do not require prior activation with antigens or lectins to undergo TCGF-induced proliferation. Furthermore, neoplastic T-cell lines originating from such patients may constitutively produce TCGF, TCGF receptors and HTLV virions³. HTLV is transmissible from cell to cell, and the infection of human T cells *in vitro* is associated with the expression of TCGF receptors⁶, which can be identified by the monoclonal antibody termed anti-Tac⁷⁻⁹. In our experience to date, T-cell populations that produce HTLV without exception also express epitopes found on TCGF receptors. Recognition of an association between HTLV virions and the Tac antigen would have clinical and theoretical implications. We now present evidence that during the replication or release of HTLV, the virion becomes preferentially associated with the Tac antigen.

As previously reported⁷⁻⁹, the 55,000-molecular weight glycoprotein detected by the monoclonal antibody anti-Tac is a normal T-cell activation marker that is identical or very closely linked to the receptor for TCGF. We have detected the Tac antigen in preparations of the neoplastic T-cell line HUT-102-

B2 derived from a T-cell lymphoma patient C.R. and in pelleted virus (HTLV_{CR}), which is released by this line, using anti-Tac-containing ascites and an enzyme-linked immunosorbent assay (ELISA). As shown in Fig. 1, the anti-Tac antibody readily bound to the purified (double-banded) HTLV preparation over a range of dilutions, while equivalent concentrations of control murine ascites did not. A similar pattern of binding was observed when a preparation of HUT-102-B2 cells was assayed. By contrast, the anti-Tac antibody bound neither to an Epstein-Barr virus-transformed B-cell line (CR-B) derived from the same patient⁴, nor to the T-cell line HUT-78, which did not release HTLV.

We next investigated whether TCGF-containing culture media could block the binding of anti-Tac to preparations of HTLV_{CR} and HUT-102-B2, as well as to phytohaemagglutinin (PHA)-stimulated peripheral blood lymphocytes from a normal donor (Fig. 2). The results show a comparable blocking effect in all three cases. Moreover, a highly purified preparation of TCGF blocked the binding of anti-Tac antibody to HTLV_{CR} (Table 1). The data, taken together, are compatible with the view that the Tac marker detected in association with the HTLV preparations represents a functional receptor for TCGF. These results prompted us to compare the relative enrichment of other known T-cell differentiation antigens which can be expressed by cells infected with HTLV.

The binding of several monoclonal antibodies served as a probe for assessing the relative enrichment of T-cell surface differentiation antigens on standardized preparations of HTLV and HTLV-producing cells. These studies relied on an internal standard of reference, in which the expression of an antigen in the HTLV preparation was compared with that of the same antigen in the HUT-102-B2 preparation in the same conditions. We found that the Tac antigen was preferentially expressed in the virus preparation relative to OKT4 (a marker on the inducer T-cell subset), LyT3 (the sheep erythrocyte rosette receptor) and OKT9 (the transferrin receptor) (Fig. 3). The same results were obtained using another HTLV_{CR} preparation purified independently of that tested in Fig. 3. Comparable results were also obtained using another virus preparation (HTLV_{C10/MJ-2}) which was derived from a different patient with an HTLV-associated T-cell lymphoma, then transmitted to normal cord blood cells and propagated in secondary cultures before paired assays of virus and virus-producing cord cells were done¹⁰.

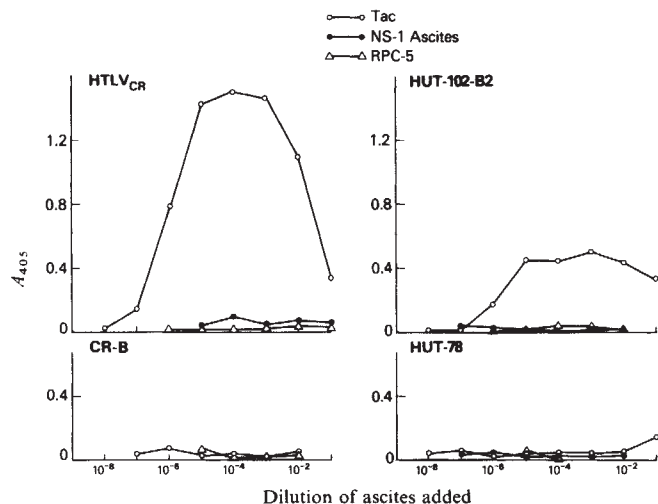


Fig. 1 Specific binding of the monoclonal antibody anti-Tac to the human retrovirus HTLV_{CR} and the cloned T-cell tumour cell line (HUT-102-B2) which gave rise to the virus. Binding of the anti-Tac reagent was observed when the HTLV preparation and HUT-102-B2 cells were used (upper panels). Antibody failed to bind when CR-B, a B-cell syngeneic to HUT-102-B2, was used, or when an unrelated T-cell line (HUT-78), which does not release HTLV, was used (lower panels).

Methods: HTLV_{CR} was collected by centrifugation of cell-free media from the HUT-102-B2 line. Purification was accomplished using zonal ultracentrifugation with RNase-free sucrose gradient (density 1.14–1.18). Electron microscopy, protein content and reverse transcriptase assays confirmed the purification procedure^{3,34}. The virus preparation was negative for 5' nucleotidase and cellular DNA polymerase. Finally the virus preparation was disrupted and lysed by 0.5% NP40 in phosphate-buffered saline (PBS). For the ELISA³⁵, 0.1 ml of virus preparation (10 µg ml⁻¹) in carbonate buffer (pH 9.6) was applied to flat-bottomed Immulon microtitre plates (Dynatech) overnight at 4 °C. Whole cells used for assay were washed three times with PBS. 0.1 ml of a standardized cell suspension (containing 7 µg of protein) was applied to each well, pelleted and desiccated. Plates were washed four times by semi-automated washer (Dynatech) using PBS, 0.05% Tween 20 and 0.02% sodium azide. 0.05 ml of anti-Tac (an IgG2a class antibody) or control NS-1 and RPC-5 (an IgG2a paraprotein-containing ascites) diluted in RPMI-5% fetal calf serum (FCS) was added to duplicate wells, incubated at 4 °C overnight and followed by four washes. 0.05 ml of goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma) diluted 1:1,000 in PBS-Tween-1% FCS was added for 2 h followed by six washings. 0.1 ml of 1 mg ml⁻¹ *p*-nitrophenol phosphate (Sigma) in diethanolamine buffer (pH 9.8) was added to each well. Plates were read after 1 h at an absorbance of 405 nm by Titertek Multiskan ELISA reader (Flow Laboratories). A Wang 2200 computer connected to the reader averaged the duplicates and subtracted the nonspecific binding. These techniques of coating the microtitre plates and performing the ELISA were used throughout these studies. The results were highly reproducible with less than 10% difference between duplicates.

To analyse the preferential association of the Tac antigen with this human retrovirus further, we used a competitive binding assay, and referred to two additional normal differentiation antigens (HLA-DR and Leu-1) which may be detected on HTLV-infected T cells in culture^{4,10}. HLA-DR, found on normal B cells, macrophages and activated T cells, is a class II histocompatibility antigen. The marker detected by Leu-1 (also referred to as T101 and OKT1) represents a p67 antigen found on most circulating human T cells. Using the ELISA technique, we tested the capacity of solubilized HTLV_{CR} and HUT-102-B2 starting cell preparations to block competitively the binding of monoclonal antibodies reactive against Tac, HLA-DR and Leu-1 to their respective ligands on HUT-102-B2 target cells on plastic (Fig. 4). The enrichment of a given antigen in the virus preparation versus the cellular preparation can be estimated by the position of the solubilized HTLV competition curve relative

Table 1 Purified TCGF blocks the binding of anti-Tac to HTLV

	³ H-anti-Tac bound (c.p.m.)
Medium	2,133 ± 205
Unlabelled anti-Tac (100 µg)	412 ± 56
Purified TCGF (µg) 4	404 ± 137
2	895 ± 188
1	936 ± 3
0.5	1,071 ± 103
0.25	1,066 ± 94
0.016	1,544 ± 76

HTLV-coated microtitre wells, prepared as described in Fig. 1 legend, were incubated for 2 h at room temperature with 15 ng of purified ³H-anti-Tac (75,000 c.p.m. per well) antibody⁹ in the presence of medium alone, 100 µg per well of purified unlabelled anti-Tac antibody as the positive control for a blocking effect, or varying amounts of purified TCGF (0.016–4 µg per well). This preparation of TCGF reveals a single spot when analysed by two-dimensional gels³³. Incubations were performed in a total volume of 200 µl of binding medium (RPMI 1640, 1 mg ml⁻¹ human IgG, 10 mg ml⁻¹ bovine serum albumin, 10 mM HEPES pH 7.4). Following serial washing to remove unbound ligand, 200 µl of 2% SDS were added to each well. Each plate was then heated to 90 °C for 1.5 min, and the samples were then transferred to vials for liquid scintillation counting. The data shown represent the mean ± s.e.m. for triplicate determinations.

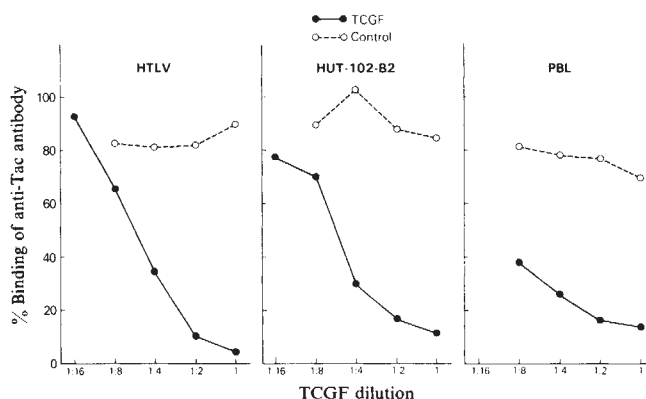


Fig. 2 T-cell growth factor-containing medium blocks the binding of anti-Tac to HTLV_{CR}, HUT-102-B2 and PHA-stimulated lymphocytes (PBL). Assays were performed as described in Fig. 1 legend, using 2×10^{-6} dilution of anti-Tac mixed with serial dilutions of a culture medium containing TCGF (Cellular Products), or control supernatant of unstimulated peripheral blood lymphocytes to which PHA was added as a last step. Peripheral blood lymphocytes from a normal donor were incubated for 3 days with 10 µg ml⁻¹ of PHA followed by six washings before desiccation on the plate (see also Table 1).

to the solubilized HUT-102-B2 cell curve. When there is sufficient enrichment of an antigen on the virus relative to the starting cell which gave rise to the virus, the competition curve with the virus will be to the left of that with solubilized HUT-102-B2 cells. The competitive blocking assays shown in Fig. 4 illustrate the enrichment of Tac antigen in the virus preparation compared with the HLA-DR and Leu-1 antigens. The amount of solubilized virus preparation needed to cause 50% inhibition of the binding of anti-Tac to its ligand was about one-sixth that of the solubilized HUT-102-B2 starting cell preparation needed for a comparable blocking effect (1.2 and 7 µg ml⁻¹, respectively), indicating that the Tac antigen was present in relatively greater amounts in the HTLV preparation than in the preparation of cells which secreted the retrovirus. On the other hand, compared with the HUT-102-B2 preparation, 3 and 10 times as much solubilized HTLV preparation were required in the

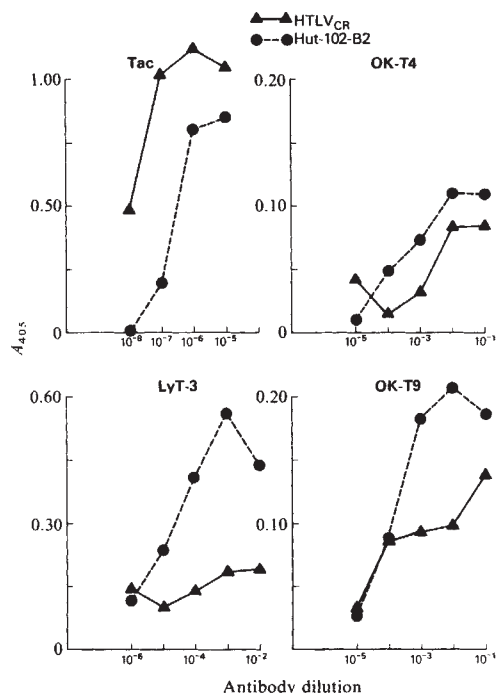


Fig. 3 Comparison of the expression of T-cell differentiation antigens associated with HTLV_{CR} and HUT-102-B2. Assays were performed as described in Fig. 1 legend using plates coated with HUT-102-B2 cells or HTLV_{CR}. Monoclonal antibodies OKT4 (directed against an antigen on the inducer T-cell subset) and OKT9 (directed against the transferrin receptor) were obtained from Ortho Pharmaceuticals. Monoclonal antibody LyT3 (directed against the sheep red cell rosette receptor) was obtained from NEN. Antibodies were diluted in RPMI media containing 5% FCS before use.

HLA-DR and Leu-1 systems, respectively, to achieve a 50% inhibition of binding by the appropriate monoclonal antibody. This indicates that these latter antigens were expressed in proportionately smaller amounts in the HTLV preparation than in the starting cell preparation. We stress that these studies allow conclusions regarding only the relative enrichment of an antigen on the HTLV preparation versus the preparation of cells which released the virus.

It is theoretically possible that these results reflect a process of selective proteolysis during the purification of HTLV. However, as expected, the competitive binding assay revealed that the virus preparation was enriched with respect to the p19 structural protein encoded by HTLV¹¹ (Fig. 4, lower right panel). We believe this militates against selective proteolysis (sparing Tac but affecting other markers tested) as the explanation for the association of the Tac antigen with HTLV described in the present studies.

In animals, enveloped viruses can incorporate or become associated with many cell-surface components¹²⁻²¹, sometimes in very high concentrations. For example, Scheinberg and Strand²¹ have described a gp55 rat cell membrane glycoprotein which is found in concentrations up to 100-fold higher in rodent retroviruses than in cells. To our knowledge, the preferential association between a retrovirus and a marker of a cell receptor for a growth factor has not been reported. The present observations suggest that Tac antigen, which is very closely linked or identical to the receptor for TCGF, is preferentially expressed on HTLV relative to other markers of T-cell differentiation. One may speculate on the biological significance of the association of this marker for a T-cell trophic factor receptor with the virus. Perhaps the Tac antigen is topographically restricted to regions of the cell membrane which are involved in the budding process by which infected cells release the retrovirus. It is clear from several viral systems^{13-15,22-24} that the budding

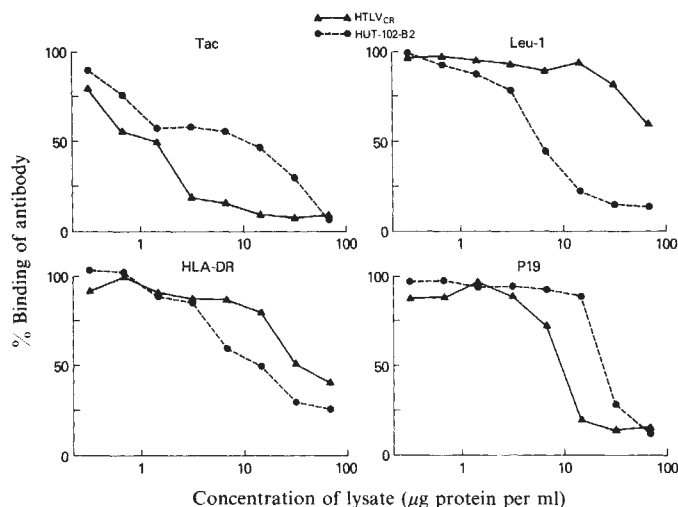


Fig. 4 Preferential enrichment of the Tac antigen on HTLV_{CR} compared with the starting HUT-102-B2 cells. Competitive inhibition of the binding of anti-Tac, HLA-DR, Leu-1 and p19 HTLV-protein monoclonal antibodies to their respective ligands on HUT-102-B2 using an ELISA technique (see Fig. 1 legend) was accomplished by prior incubation of the monoclonal antibody with the solubilized lysates of HUT-102-B2 or HTLV_{CR} at various concentrations for 2 h at room temperature before loading on the target microtitre plate. To ensure appropriate sensitivity in the competition assay, the amount of monoclonal antibody binding used as a reference for the blocking studies was set at ~50% of the amount which yielded maximal absorbance in preliminary ELISA testing with no competitors. Solubilized HTLV_{CR} was prepared as described in Fig. 1 legend. A solubilized HUT-102-B2 preparation was made by washing HUT-102-B2 cells five times in PBS, then suspending them in PBS containing 0.5% NP40 for 15 min at 4 °C. The nuclear fraction was removed by pelleting at 13,000 r.p.m. using a microfuge (Beckman Instruments). Enrichment of the Tac antigen is illustrated by a leftward position of the HTLV_{CR}-solubilized lysate curve in reference to the curve of comparably treated HUT-102-B2. As expected, a similar leftward position was demonstrated for the p19 HTLV structural protein, suggesting that selective proteolysis during virus purification is not the explanation for the relative enrichment of the Tac antigen in these assays.

process is not a random event, but rather the complex integration of specific virally encoded polypeptides and nucleic acids into a structural unit, leading to the release of extracellular virions. The transition of virus-specific glycoprotein precursors in the intracellular membrane system to a plasma membrane maturation complex involves many post-translational alterations to produce polypeptides ready for incorporation into the budding site^{15,24,25}. The Tac antigen might be linked to these events.

It is also possible that the association of the Tac antigen with HTLV is important for the primary induction of the transformed state in T lymphocytes. Interaction between a cell and a virus particle at the surface level is, of course, one of the essential initial steps in viral infection of cells (reviewed in refs 26, 27). Human cells susceptible to infection by retroviruses may have a class of surface receptors that mediates the adsorption and penetration of the virus particle. The Tac antigen might belong to this class, and it is conceivable that HTLV infection expands a small subset of T cells that express the Tac antigen at the time of initial contact with the virus. In keeping with this, enveloped viruses in animals have specific receptors on cells that are susceptible to infection by these viruses²⁶⁻³⁰. Specific binding sites of enveloped viruses on cell surfaces have frequently been visualized by electron microscopy near the sites of virus budding³¹. Binding sites specific for thymotropic murine leukaemia viruses were found in high concentration in thymic lymphoma cell lines induced by this class of virus but were hardly detectable on several non-T leukaemias, plasmacytomas and normal thymocytes or spleen cells²⁹. Of interest in regard to the present

studies, Cogniaux *et al.*³² analysed several monoclonal antibodies produced by murine hybridomas after immunization with semi-purified baboon endogenous virus (BaEV). Two monoclonal antibodies tested appeared to react with cellular epitopes localized on or near the receptors for BaEV on human cells. Finally, HTLV may have evolved the gene(s) encoding antigenic determinants which cross-react with the Tac antigen, thereby conferring an as yet undefined selective advantage on the virus in its replication and transmission in man. These will be topics for further research.

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Tightly associated lipids may anchor SV40 large T antigen in plasma membrane

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Simian virus 40 (SV40) large T antigen, a multifunctional protein necessary for lytic growth and cell transformation¹, is located mainly in the nucleus² and in small amounts on the cell surface (surface T)^{3–5}. Surface T may have a passive role in SV40 tumour rejection by cytotoxic T cells as a component of SV40-TSTA (tumour-specific transplantation antigen)⁶. The unusual induction of this immune response by immunizing mice with soluble T antigen⁷ led us to investigate the *in vitro* binding of T antigen to the surface of living cells in more detail^{8,9}. Our results show that native surface T and a minor subset of large

T antigen having a high cell surface binding affinity *in vitro*, behave like integral membrane proteins¹⁰. Several viral proteins^{10–13} including SV40 T antigen¹⁴ and cellular proteins¹⁵ seem to be linked to fatty acids (acylation). To analyse whether this mechanism is involved in the stable attachment of *in vitro*-bound T antigen to the plasma membrane of living target cells, we determined the degree of labelling of this molecule by using target cells prelabelled with ³H-fatty acid. Here we report that T antigen extracted from unlabelled SV40-transformed cells (SV80) becomes ³H-labelled after *in vitro* binding to the cell surface of ³H-palmitate-prelabelled HeLa cells. These results suggest that T antigen attached externally to living cells, may be anchored by tightly linked lipids.

Most of the acylated viral proteins are imbedded in the membrane via a hydrophobic region^{10–12}. However, the predicted amino acid sequence¹⁶ indicated no hydrophobic segments in the polypeptide chain of T antigen. To test whether a tight linkage to lipids is involved in the stable anchorage of T antigen in the plasma membrane, we analysed the association of T antigen with lipids in SV40-transformed human cells (SV80). SV80 cells (2×10^6) that had been labelled metabolically with ³⁵S-methionine or ³H-palmitate were extracted with lysis buffer, which can solubilize radioiodinated surface T³. T antigen was immunoprecipitated and analysed as described in Fig. 1 legend. Figure 1A shows that ³H-labelled T antigen co-migrated with intact ³⁵S-methionine-labelled T antigen (Fig. 1B) corresponding to a molecular weight (M_r) of ~90,000. Confirming recent reports¹⁴, our results indicate that T antigen can become tightly bound to fatty acids, as has been reported previously for several other viral proteins^{10–13}, and for the human transferrin receptor¹⁵.

Our previous observations indicated that there exists a minor subclass of T antigen having a tight cell surface binding affinity and with physicochemical properties of native surface T, suggesting that in both cases T antigen behaved like an integral membrane protein⁹. To test in greater detail whether a tight association with lipids participates in this stable anchorage of *in vitro* cell surface-bound T antigen, we extracted unlabelled 2×10^8 SV80 cells with 0.9 M hydroxylamine (pH 7) to solubilize acylated and membrane-bound T antigen by cleavage of esterified fatty acids (see ref. 17). After dialysis of the hydroxylamine extract against growth medium it was incubated for 1 h at 37 °C with 2×10^6 intact monolayer cells (HeLa) which had been prelabelled with ³H-palmitate and washed to remove excess ³H-palmitate as described in Fig. 2 legend. These cells were then incubated with extraction buffer and analysed for ³H-labelled T antigen by immunoprecipitation using a mixture of media containing monoclonal antibodies (given by E. Harlow, ICRF, London; see ref. 18) directed against an N-terminal (PAb 430; 0.25 ml) and a C-terminal (PAb 405; 0.25 ml) region of T antigen. In control experiments this mixture was adjusted to immunoprecipitate the total amount of T antigen present in 5×10^6 SV80 cells or SV40-infected monkey cells (TC-7). Figure 2 shows that the monoclonal antibodies PAb 405 and PAb 430 specifically precipitated a ³H-labelled molecule which co-migrated with T antigen.

An *in vitro* conversion of ³H-labelled fatty acids into amino acids or molecules linked to proteins (sugar, nucleotides, etc.) is usually not significant¹². In our *in vitro* conditions only a minor subclass of T antigen obtained by hydroxylamine treatment, but not by homogenization of donor cells, became tightly associated with ³H-labelled lipids of the target cells. These observations considerably reduce the possibilities of conversion of ³H-palmitic acid into other strongly binding components. To exclude both nonspecific labelling and the metabolic labelling of T antigen-related cellular target proteins¹⁹ we performed several control experiments: (1) In the absence of monoclonal antibodies no ³H-labelled protein was precipitated. (2) Immunoprecipitation with PAb 405/430 of extracts of ³H-palmitate-labelled HeLa cells which had either not been incubated with T antigen-containing cell extracts or incubated at 4 °C, did not detect any ³H-labelled protein (data not shown).

Fig. 1 SV40 large T antigen is bound to lipid as shown by ^3H -palmitic acid labelling of SV40-transformed SV80 cells. 2×10^6 SV80 cells grown in one Petri dish in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS) were labelled with: A, 1 mCi ^3H -palmitic acid ($10\text{--}30 \text{ Ci mmol}^{-1}$; NEN) or B, 30 μCi ^{35}S -methionine (800 Ci mmol^{-1} ; NEN) for 4 h at 37°C . Immunoprecipitated T antigen was analysed on a 10% SDS-polyacrylamide gel. ●—●, Hamster SV40 tumour serum (HT); *—*, normal hamster serum (NHS). Molecular weight markers were run on a separate gel: phosphorylase A (M_r 94,000), bovine serum albumin (M_r 68,000) and ovalbumin (M_r 43,000).

Methods: ^3H -palmitic acid was air-dried, dissolved in 100 μl dialysed FCS by sonication and added to 1 ml of DMEM. After labelling the cells were washed three times with DMEM, scraped off the dishes and extracted with 500 μl lysis buffer (100 mM Tris-HCl pH 7, 100 mM NaCl containing 1% Nonidet P40, NP40) for 30 min on ice, then centrifuged at $105,000g$ for 30 min. The supernatant was cleared by immunoprecipitation with 10 μl NHS and 200 μl of 10% (w/v) *Staphylococcus aureus* suspension as described by Kessler²⁰ at 4°C for 1 h. After centrifugation, T antigen was immunoprecipitated from the supernatant with 10 μl HT and 200 μl *S. aureus* overnight at 4°C . The immunoprecipitates were washed five times with NET buffer (150 mM NaCl, 5 mM EDTA, 1% sucrose, 1% NP40, 50 mM Tris-HCl, pH 7.3) and finally with 100 mM Tris-HCl, pH 6.8. The immunoprecipitates were eluted in sample buffer (1% SDS, 700 mM mercaptoethanol, 0.01% bromophenol blue, 10% glycerol, 650 mM Tris-HCl pH 6.8) and boiled for 10 min. *S. aureus* were spun down and the supernatants were run on 10% SDS-polyacrylamide gels (10 cm) according to Laemmli²¹ in glass tubes (5 mm diameter). After electrophoresis the gels were cut into 2-mm slices, eluted with 200 μl water and counted for radioactivity in 2 ml of Quickszint (Zinsser, Frankfurt).

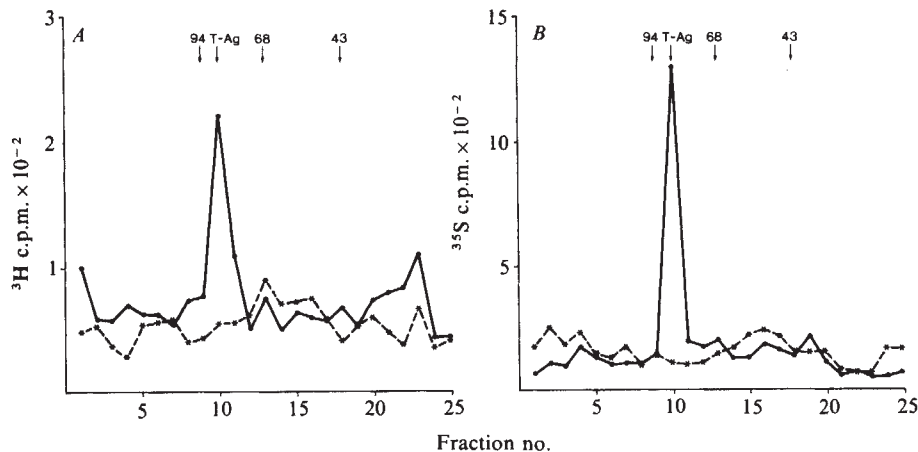
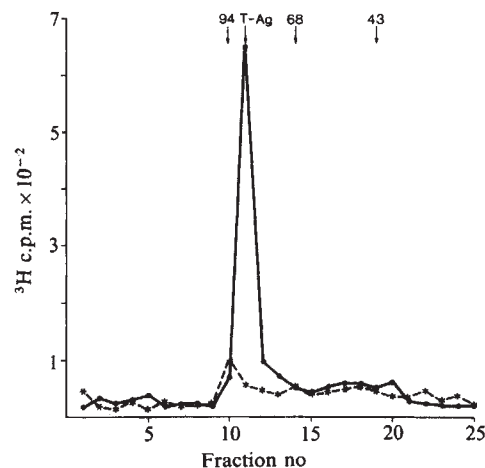


Fig. 2 Solubilized T antigen became bound to cellular lipid after incubation with ^3H -palmitic acid-prelabelled target cells. ●—●, Immunoprecipitation with monoclonal antibodies PAb 405/430; *—*, immunoprecipitation with normal mouse serum. The immunoprecipitates were analysed on 10% polyacrylamide gels as described in Fig. 1 legend, and the molecular weight markers were the same as in Fig. 1.

Methods: Unlabelled SV80 cells (2×10^8) were extracted with 2 ml of 0.9 M hydroxylamine in HEPES-buffered DMEM, pH 7, for 2 h at room temperature. After extraction the cells were spun down and the supernatant clarified by centrifugation at $105,000g$ at 4°C for 30 min. The extract was extensively dialysed against HEPES-buffered DMEM, then incubated for 2 h at 37°C with 2×10^6 HeLa cells grown in a Petri dish prelabelled with 1.5 mCi ^3H -palmitic acid for 16 h. After incubation of the extract, the target cells were washed at least 10 times with HEPES-buffered DMEM to remove nonspecific cell surface-attached proteins. The cells were collected mechanically and extracted as described in Fig. 1 legend. Immunoprecipitation was done first for 1 h at 4°C with 10 μl of normal mouse serum, 10 μl of purified rabbit anti-mouse immunoglobulin (1 mg ml^{-1} ; Nordic, Byk Mallinckrodt) and 200 μl 10% *S. aureus*, followed by immunoprecipitation of T antigen at 4°C overnight with 500 μl of a 1:1 mixture of monoclonal antibodies against T antigen (PAb 405 and 430; see ref. 18), 10 μl of rabbit anti-mouse immunoglobulin and 200 μl of a 10% *S. aureus* suspension.



(3) Mixing 200- μl aliquots of T antigen-containing cell extracts (10^8 cells ml^{-1}) with 1 mCi of ^3H -palmitate did not produce labelling of T antigen with ^3H (data not shown). In general, the strongly denaturing conditions of SDS-polyacrylamide gel electrophoresis dissociate small molecules and macromolecules bound to proteins in native conditions. We also did not detect any label in T antigen peaks on SDS gels after incubation of cell extracts with ^3H -labelled amino acids or mononucleotides (unpublished).

Thus, our data clearly indicate that *in vitro* cell surface-bound T antigen can bind tightly to ^3H -palmitate. Therefore, it is possible that T antigen, concurrent with or shortly after binding to the cell surface, becomes stably attached to the plasma membrane via tightly bound lipids. This raises the possibility that acylation of externally attached proteins may serve to stabilize attachment by anchorage in the plasma membrane.

Although our previous experiments using immunofluorescence microscopy indicated the cell surface localization of *in*

vitro-bound T antigen⁹, at present we cannot exclude the possibility of intracellular acylation during uptake and recycling of cell surface-bound T antigen. However, despite our lack of knowledge concerning the mechanism of attachment of proteins to membranes, the present data support the notion that the cell surface-binding subspecies of T antigen may be anchored in the plasma membrane via tightly bound lipids.

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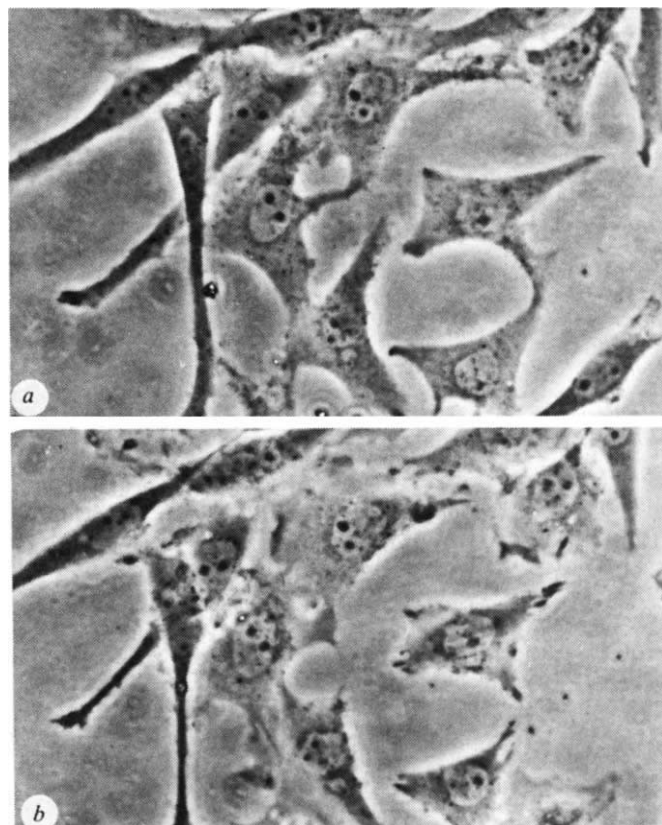


Fig. 1 Phase contrast micrograph of CHO cells before and after microinjection. *a*, Phase contrast photograph of a field of CHO cells immediately before microinjection; *b*, photograph of the same cells 3 h after injection with phosphocellulose-purified porcine tubulin (the cells were labelled with ^{35}S -methionine during the last hour). Cell viability, as determined by the maintenance of an unaltered phase contrast image and by the ability of the cells to incorporate ^{35}S -methionine into new protein, is not affected by the microinjection procedure.

Elevation of tubulin levels by microinjection suppresses new tubulin synthesis

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Most eukaryotic cells rapidly and specifically depress synthesis of α - and β -tubulin polypeptides in response to microtubule inhibitors which cause microtubule depolymerization and presumably increase the intracellular concentration of free subunits^{1–4}. Other drugs which interfere with microtubule function but which lead to a decrease in the subunit pool size have little effect on the rate of new tubulin synthesis^{1,2}. These findings have previously been interpreted to indicate that cultured cells synthesize tubulin constitutively unless the subunit pool rises above a specified level. At this point an autoregulatory control mechanism is triggered which suppresses new tubulin synthesis through specific loss of tubulin mRNAs^{2,4}. That tubulin RNA levels are dramatically lowered by microtubule depolymerizing drugs is unquestionably correct; that fluctuations in the depolymerized tubulin pool size are responsible for altered RNA levels rests, however, entirely on the presumptive effects of different microtubule drugs. This caveat is not trivial, as these drugs induce gross morphological alterations, and the specificities and detailed mechanisms of action of such drugs remain poorly understood. To investigate the effect of altered levels of tubulin subunits on the rate of new tubulin synthesis in mammalian cells, we have microinjected purified tubulin subunits into cells in culture and analysed the synthesized proteins. We report here that tubulin synthesis is rapidly and specifically suppressed by injection of an amount of tubulin roughly equivalent to 25–50% of the amount initially present in the cell, thus indicating the presence of an eukaryotic, autoregulatory control mechanism which specifies tubulin content in a cultured mammalian cell line.

To demonstrate the feasibility of using microinjection to test whether tubulin synthesis could be modulated by exogenously derived subunits, cultured Chinese hamster ovary (CHO) cells were grown in small wells rimmed with silicon grease or on small fragments of a glass coverslip. Using either approach, a single group of cells (that is, all cells within a single well or all attached to a particular cover chip) could be injected sequentially and then labelled with ^{35}S -methionine. For the microwells, such labelling was conveniently achieved by replacement of the well culture medium with ^{35}S -methionine-containing medium;

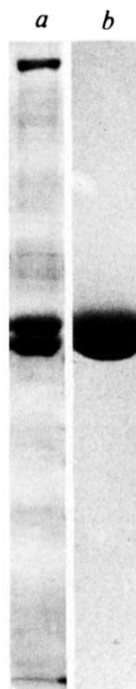
for the chips, labelling was performed after rinsing the chip through a dish of methionine-free medium, followed by placement of the chip into a bead of ^{35}S -methionine containing medium which was resting on a piece of parafilm in a humidified chamber. Using either procedure, 300–1,200 c.p.m. could be incorporated per cell in a 1-h labelling period. Moreover, no major morphological changes could be observed as a result of the microinjection or labelling manipulations.

A demonstration of these findings is shown in Fig. 1, which displays phase contrast photographs of a group of CHO cells grown on a cover chip. Figure 1*a* shows the cells before injection and Fig. 1*b* shows the same cells 3 h after injection, during the last hour of which they were labelled with ^{35}S -methionine as detailed above. Microtubule protein for microinjection was purified from hamster brain and from porcine brain by three cycles of assembly/disassembly. An SDS-polyacrylamide gel of the resultant hamster microtubule protein in which α - and β -tubulin are the principal components (~80% of total protein) is shown in Fig. 2*a*. Microtubule-associated proteins, including a prominent high molecular weight doublet, are also present in this preparation. More highly purified porcine brain tubulin was obtained by chromatography of hog brain microtubule protein on phosphocellulose⁵. By Coomassie blue staining, the tubulin obtained (shown in Fig. 2*b*) contains no proteins other than the α - and β -tubulin polypeptides.

To determine whether the level of tubulin synthesis could be specifically depressed by injection of tubulin, we microinjected

Fig. 2 Polyacrylamide gel electrophoretic analysis of protein samples for microinjection. *a*, Hamster brain microtubule protein purified through three cycles of assembly, visualized by staining with Coomassie brilliant blue following electrophoresis on an 8.5% SDS-polyacrylamide gel¹³; *b*, gel electrophoresis of porcine brain tubulin purified through three cycles of assembly/disassembly followed by chromatography on phosphocellulose to remove all microtubule associated proteins.

Methods: Microtubule protein, containing tubulin and its associated proteins, was prepared from hamster or porcine brain by three cycles of polymerization/depolymerization using a modification of the method of Shelanski *et al.*¹⁴. Brain tissue (1 g per ml of buffer) was homogenized in cold protein buffer (PB; 50 mM PIPES pH 6.9, 1 mM EGTA, 0.1 mM EDTA, 0.5 mM MgCl₂, 1 mM 2-mercaptoethanol, 1 mM GTP). Following centrifugation at 4 °C for 30 min at 45,000 r.p.m. in a Beckman 50 Ti or 50.2 Ti rotor, the supernatant was made 4 M in glycerol by addition of an equal volume of PB containing 8 M glycerol, warmed at 37 °C for 20 min, and the polymerized microtubules pelleted by centrifugation at 25 °C (45,000 r.p.m. for 30 min). The pellet was then resuspended in cold PB and a second complete cycle of disassembly/assembly was performed as before with the exception that glycerol was omitted from the succeeding polymerization step. The resultant two-cycle microtubule protein was stored by freezing microtubule pellets in liquid nitrogen and storage at -80 °C. Before use for microinjection, the thawed microtubule protein was subjected to a third complete cycle of polymerization/depolymerization. For protein to be chromatographed on phosphocellulose⁵, the third-cycle microtubule pellet was resuspended in ice cold 25 mM PIPES pH 6.9, 0.1 mM EDTA, 0.5 mM MgCl₂ and applied to a pre-equilibrated phosphocellulose column (Whatman, P11). 3 mg of protein were loaded per ml of resin. The tubulin, which does not absorb to the column in these conditions, was eluted and brought to 50 mM PIPES, 0.5 mM MgCl₂ and quickly frozen in small aliquots in liquid nitrogen.



a miniwell containing about 15 CHO cells with depolymerized hamster brain microtubule protein (Fig. 2*a*) at an initial concentration of $\sim 15 \text{ mg ml}^{-1}$. This protein solution, which was difficult to inject because aggregation occurs rapidly and clogs the needle, was initially selected on the basis of its species identity with respect to the CHO cells. Immediately following injection, the cells were returned to an incubator for 2 h. At this point, ³⁵S-methionine was added and newly synthesized proteins were pulse-labelled for 1 h. Total cell protein was analysed by two-dimensional gel electrophoresis. A fluorograph of the resultant experiment is shown in Fig. 3*c*, along with the corresponding experiments showing proteins synthesized by cells which were not injected (Fig. 3*a*) or which were injected with the microtubule buffer only (Fig. 3*b*). The position of α -tubulin (a faint doublet just to the basic side of the vimentin spot) and the more prominent β -tubulin polypeptide are indicated by the labelled arrows in the figure. The spots corresponding to the tubulin subunits were identified unambiguously by two-dimensional gel analysis of immunoprecipitates obtained with an antibody against both subunits². Although the α -tubulin and vimentin spots are poorly separated so that comparison of the relative amount of α -tubulin synthesis in the control and tubulin-injected cells is difficult, it is readily apparent that β -tubulin is diminished in intensity in the tubulin-injected sample (Fig. 3*c*).

Moreover, this suppression can be even more dramatically demonstrated by injection of completely purified tubulin sub-

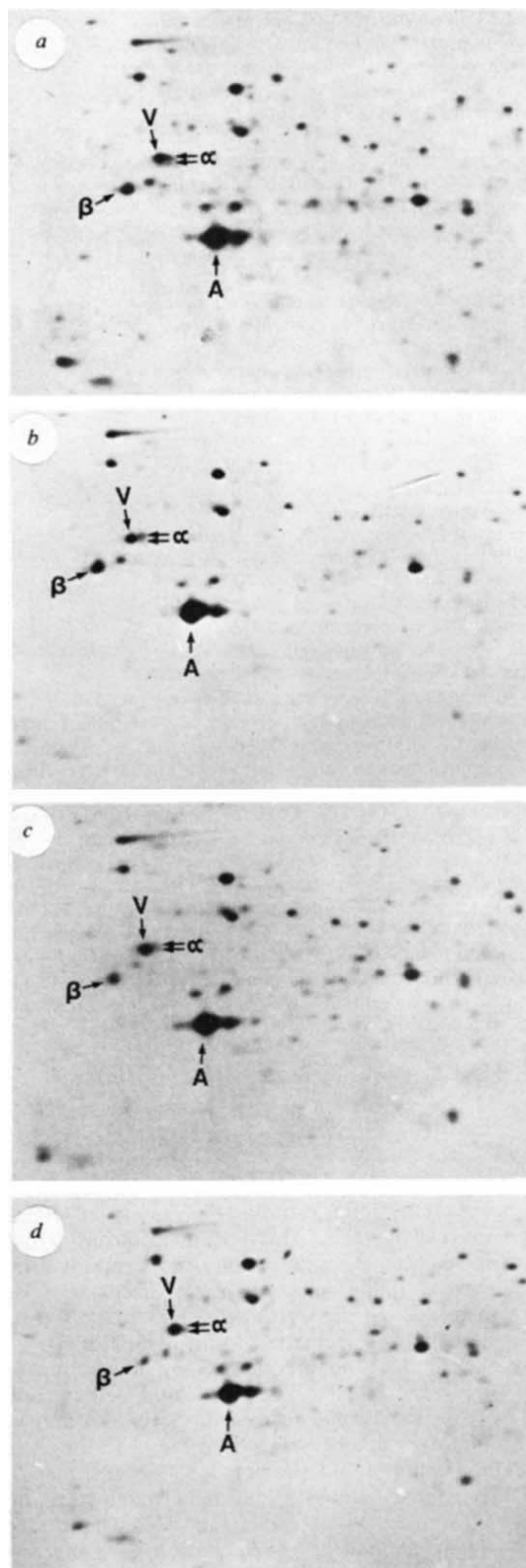
units derived from a nonhomologous species. Figure 3*d* displays the pattern of protein synthesis in response to injection of CHO cells with a 6.6 mg ml^{-1} solution of purified porcine brain α - and β -tubulin. β -Tubulin synthesis is dramatically lowered compared with that of the initial level (compare Fig. 3*d* with Fig. 3*a* and *b*) and α -tubulin synthesis is also apparently depressed several-fold. For quantitation of the depression in β -tubulin synthesis, the portions of the dried gels corresponding to the β -tubulin spot, the actin spots and one other prominent spot of unknown identity were excised (using the fluorographs as guides) and the radioactivity was measured by liquid scintillation counting. Although the number of counts was low even in the control sample (~ 200 for β -tubulin), only 1/10th of this level was present in the β -tubulin spot from the tubulin-injected sample (after normalizing the actin and unknown spot radioactivities in the two samples). In addition, injection of a parallel aliquot of tubulin which had been pretreated with colchicine ($150 \mu\text{M}$ colchicine for 10 min at 37 °C followed by removal of unbound colchicine by centrifuge desalting on Biogel P6) also induced specific suppression of β -tubulin synthesis, although in two separate experiments the effect was slightly less dramatic than that with the untreated tubulin. This suppression appears specific to injected tubulin subunits, as injection of preimmune IgG or bovine serum albumin even at substantially higher concentrations (up to 50 mg ml^{-1}) induces no detectable alterations in cellular protein synthesis (J.R.F., unpublished).

Tubulin comprises roughly 2–3% of total cell protein in most cultured mammalian cells⁶. Its intracellular concentration has been estimated to be about 2 mg ml^{-1} (ref. 6), of which $\sim 50\%$ is in the unpolymerized form at any specific time^{6–8}. The volume routinely injectable into cultured cells without loss of viability has been empirically determined by previous workers to be approximately 1/10th of the initial cell volume^{9,10}. Thus, injection of exogenous, depolymerized tubulin at a concentration of 10 mg ml^{-1} will increase the cellular tubulin content by roughly 50% and initially increase the tubulin subunit pool by 100%. Within the uncertainties of the measurements this is the same order of increase in subunit pool size as can be achieved by complete depolymerization of the endogenous microtubules with microtubule inhibitory drugs. Our finding that the microinjection of tubulin from a homologous (hamster) or heterologous (hog) source rapidly and specifically depresses new tubulin synthesis, clearly, if not unexpectedly, shows that cultured cells monitor tubulin content and adjust the rate of new synthesis accordingly.

To the interesting and important question of whether it is truly the level of tubulin subunits as opposed to the level of polymer which is regulated, the data are strongly suggestive, but not yet unambiguous. Although the injection of polymerization-competent microtubule protein did result in some suppression of tubulin synthesis, the heterologous phosphocellulose-purified tubulin was a considerably more effective inhibitor of new synthesis. The capacity of this purified protein for self-assembly into microtubules *in vitro* even in ideal polymerizing conditions is notoriously labile (see ref. 11 for example). From quantitative electron microscopy¹² (done in the presence of excess microtubule-associated proteins), we estimate that at most 30–40% of the tubulin used in these experiments was able to polymerize *in vitro* immediately before injection. The precise fraction of this tubulin that remains competent for polymerization following the high shear/high pressure injection and the fraction that actually becomes polymerized in the *in vivo* conditions is unknown. In this regard, however, tubulin subunits from the same preparation of tubulin which were pretreated with colchicine (a binding event which strongly inhibits polymerization and is essentially irreversible *in vitro*) also inhibit new synthesis, although not as effectively. This latter finding is somewhat troubling because it seems to conflict with the hypothesis that the unpolymerized subunit pool is the entity actually measured. In practice, however, given that the colchicine-treated subunits were obligatorily diluted to $\sim 70\%$ of their original concentration during the binding reaction and

Fig. 3 Two-dimensional gel analysis of newly synthesized proteins in CHO cells following microinjection. *a*, Protein synthetic pattern from uninjected cells; *b*, pattern of protein synthesis from cells injected with the microtubule PB lacking EGTA; *c*, pattern from cells microinjected with hamster brain microtubule protein at an initial concentration of $\sim 15 \text{ mg ml}^{-1}$ in PB lacking EGTA; *d*, pattern from cells microinjected with 6 mg ml^{-1} of phosphocellulose-purified porcine brain tubulin. Arrows indicate the positions of actin (*a*), α -tubulin (α), β -tubulin (β) and vimentin (*v*), respectively. Unambiguous determination of the positions of the α - and β -tubulin polypeptides was achieved by two-dimensional analysis of immunoprecipitated proteins using an antibody to α - and β -tubulin². For proteins synthesized by each aliquot of 15–20 cells during the 1-h labelling period the fluorographs shown have been normalized by varying the exposure times over 2–4 days.

Methods: CHO cells, maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum, were grown on 35-mm plastic Petri dishes on which small circular wells (2 mm diameter $\times$ 2 mm high) had been constructed with silicon grease (Dow Corning high vacuum grease). Cells were seeded such that ~ 15 –20 cells attached within each well. Microinjection was performed with fine-tipped glass needles using a modification of the technique of Graessmann⁹, as described in detail elsewhere¹⁵. Briefly, glass needles were pulled to a final tip diameter of about 0.5 μm . Before injection, each protein solution was centrifuged for 5 min at 100,000 *g* in an Airfuge (Beckman) to pellet aggregated material which might clog the needles. The solution was then loaded into a needle by suction with a syringe. All cells within a well were serially microinjected at room temperature ($\sim 20^\circ\text{C}$) using syringe pressure to control the volume injected and the cells were immediately returned to a 37°C , CO_2 incubator. Injection of each aliquot required ~ 15 min. After 2 h, each dish was removed and all medium outside a well removed by aspiration. The medium from inside a well was then carefully removed and replaced with two successive washes of methionine-free DMEM. Finally, ^{35}S -methionine ($\sim 1,000 \text{ mmol}^{-1}$, NEN) was lyophilized and resuspended at 10 mCi ml^{-1} in methionine-free DMEM. 5 μl of this labelling medium were added to each well and the cells were returned to the incubator for 1 h. The ^{35}S -methionine-containing medium was then removed and the cells gently washed twice with phosphate-buffered saline. Total cell protein was solubilized in 5 μl of Laemmli gel sample buffer¹⁴ (containing 2% SDS, 100 mM dithiothreitol) and fully denatured by boiling for 2 min. Samples were then frozen in liquid nitrogen and stored at -80°C . For display of labelled proteins, the samples were thawed, diluted with 45 μl of O'Farrell lysis buffer¹⁶ containing 4% NP40, brought to 9.5 M urea by addition of solid urea, and electrophoresed on isoelectric focusing gels according to O'Farrell¹⁶ with the exception that the gels were made 2% in NP40. Ampholytes (80% 5–7, 20% 3–10) were from BioRad. After focusing for 16 h at 400 V and at 800 V for 1 h, the samples were electrophoresed in the second dimension on 8.5% polyacrylamide gels¹⁴. Radioactively labelled proteins were visualized by fluorography¹⁷.



subsequent removal of unbound drug by desalting, and given the limitations inherent in injecting each cell with a comparable amount of exogenous material, this apparent difference in efficacy is probably not significant. Thus, the current data, when combined with the earlier studies using microtubule inhibitory drugs which presumably increase the level of unassembled subunits, support the argument that the unpolymerized tubulin pool is the molecular entity which is ultimately detected by the cell.

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Opening the closed ribosome-binding site of the lysis cistron of bacteriophage MS2

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In prokaryotes gene expression is mainly regulated at the levels of transcription and translation. An important form of translational control operates at the initiation of protein synthesis. For instance the translation of an existing mRNA can be prevented by features in the mRNA structure that prohibit binding of ribosomes. This type of control is frequently applied to polycistronic mRNA to forestall translation of a downstream cistron until the 5' neighbouring cistron has been read¹⁻⁷. Such translational coupling or sequential reading also facilitates the shutting off of several cistrons from one control point⁶. An interesting example of a nontranslatable message is the lysis (L) cistron, present as an overlapping gene in the RNA bacteriophage MS2 (refs 8-10; Fig. 1). The start of the L cistron is not directly accessible to ribosomes. Instead its translation is strictly coupled to the passage of ribosomes over the preceding coat cistron². We have now analysed which features in the MS2 RNA structure deny ribosomes access to the start of the L message. We report here that small deletions, introduced about 40 nucleotides 5' to the start codon of the L gene, remove the initiation barrier and open the cistron to independent translation. An RNA secondary structure accounting for the closed state of the ribosome binding site is proposed.

We have reported previously that initiation of protein synthesis at the L cistron is only possible by a small fraction of ribosomes which, while translating the coat gene, shift their frame and terminate at either one of two out-of-phase stop codons just preceding the L cistron start codon. Termination is then followed by reinitiation of protein synthesis at the L gene².

Figure 2 shows the lysis gene (as a DNA copy) inserted in plasmid pMS1.00 downstream from the temperature-controlled promoter p_L from phage λ ¹¹. As the start of the coat gene is not present in pMS1.00, this clone does not produce the lysis protein when transcription from p_L is induced at 42 °C (refs 2, 12).

We suppose that the failure of ribosomes to initiate independently at the L cistron is due to the masking of the translational start signals by RNA secondary structure. If this is the case, it may be possible to expose these signals by the gradual removal of nucleotides located proximal to the ribosome-binding site. Accordingly, pMS1.00 was opened at its unique *EcoRI* site, treated with the exonuclease *Bal31* and recircularized in the presence of the *EcoRI* linker molecules 5' GGAATTCC. Appropriate recloning of the shortened MS2 DNA insert allows the construction of pMS1.00-derived plasmids, in which the MS2 DNA information is shortened while the p_L region is kept constant. These new clones are termed pMS1.*N*, where *N* is the number of nucleotides removed from the MS2 DNA sequence.

Selected clones were sequenced to size the deletion and their lysis phenotype was determined by induction of the p_L promoter at 42 °C. The results (Figs 3a, 4) show that it is indeed possible to open the closed cistron by introducing appropriate deletions. On deletion of 4 nucleotides (pMS1.4), expression of the L gene becomes intermediate. Deleting 2 more nucleotides (pMS1.6) leads to optimal expression. This phenotype persists for the next five clones, in which up to 26 nucleotides are removed (pMS1.6 to pMS1.26). When the deletion is extended to nucleotide 1,665 (pMS1.31), cell lysis disappears again, perhaps because of the removal of 2 nucleotides from the Shine-Dalgarno region of the L gene.

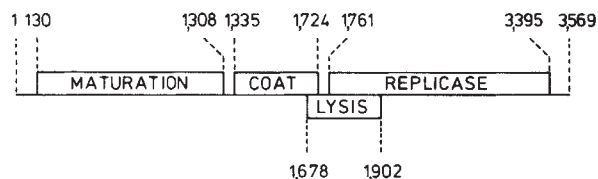


Fig. 1 Genetic map of the RNA phage MS2. Nucleotide numbers are taken from ref. 15. Coat and replicase genes are read in the 0 frame and the lysis gene in the +1 frame. Thus, if in the sequence 5' AGCU, AGC is a 0-frame codon, GCU is a +1-frame codon.

Coat-gene-independent translation of the L cistron becomes possible once the sequence GACUGC is deleted (pMS1.6; Fig. 3a). This sequence seems to play a decisive part in maintaining the closed state of the L gene. This result can be interpreted in two opposing ways: either the 6-nucleotide deletion disrupts a double-stranded structure, freeing a single-stranded ribosome recognition site; or the 6-nucleotide deletion accidentally creates an ordered RNA region which promotes the binding of ribosomes. The second interpretation is unlikely, as the chance that favourable ordered structures form in all eight *Lys*⁺ deletion mutants is very small. On the other hand, the elimination of an ordered region by the variously sized deletions shown in Fig. 3a is easily conceivable. We accept this interpretation and infer that the closed state of the L cistron is maintained by RNA secondary structure involving the sequence GACUGC. Therefore one would expect deletions left of the sequence not to expose the L cistron, which is indeed the case. The required deletions were made in pMS32.00. This plasmid is identical to pMS1.00 except that it contains the MS2 DNA region 1,221-2,057 instead of 1,628-2,057. To prevent expression of the lysis gene in the clones via coat gene translation we arranged premature termination of protein synthesis at nucleotide 1,415 (see Fig. 3b legend for details). The top four clones of Fig. 3b carrying a deletion 5' to the GACUGC sequence do not lyse. Moreover, once the sequence GACUGC is absent, the L cistron is open, irrespective of the newly created sequence (compare pMS1.18d, pMS1.28d, pMS32.32.Sal⁻ and for example, pMS1.6). Alternatively, as long as GACUGC is present the cistron is closed no matter what its 5' context [pMS1.00, pMS32(3a, 6a, 6b, 9)Sal⁻].

Figure 5 shows our proposed RNA secondary structure for the region studied, based on a Tinoco plot. In this model, the inhibitory sequence is base-paired to the Shine-Dalgarno region of the L gene. This interaction presumably accounts for the closed state of the L cistron. The structure is consistent with

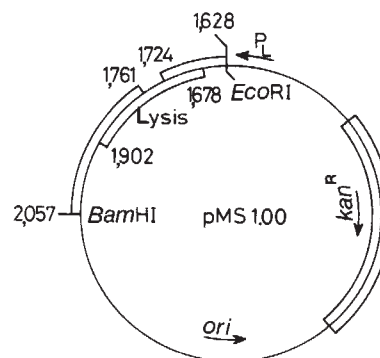


Fig. 2 Basic features of plasmid pMS1.00. This plasmid is constructed by inserting the *EcoRI*-*BamHI* segment of MS2 DNA (nucleotides 1,628-2,057) into the vector pPLa831 (ref. 11). p_L is the main leftward promoter of phage λ . The *EcoRI* and *BamHI* sites in pPLa831 are located next to each other, 113 nucleotides downstream from the transcription start point of p_L . At 28 °C this promoter is closed by the λ repressor encoded in the chromosome of the host M5219 (ref. 11) used in this study. At 42 °C the repressor (the thermolabile mutant λ cI857) is unstable and the promoter is turned on. Further details of the plasmids and strain can be found in refs 2 and 11. pMS1.00 was previously called pMS1 (ref. 2).

Fig. 3 Effect of deletions in the L-gene leader on cell lysis. *a*, The deletions shown are obtained by treating pMS1.00 (Fig. 2), opened at its unique *EcoRI* site, with exonuclease *Bal31*. Then the *EcoRI* linkers GGAATTC are attached with T4 DNA ligase. After *EcoRI* treatment to produce sticky ends, the DNA is recircularized with T4 DNA ligase. To restore the original length of p_L region, the shortened MS2 DNA section (*EcoRI*-*BamHI* fragment) is recombined in a vector that has the p_L region intact. To facilitate selection of the proper clones the recloning is done in pPLc28 (ref. 11), which carries resistance to ampicillin, but is further very similar to pPLa831, which has kanamycin resistance¹¹. Clones pMS1.18d and pMS1.28d are obtained by treating pMS1.00, opened at the *EcoRI* site, with exonuclease *Bal31* and closing with T4 DNA ligase in the absence of linker molecules. In pMS1.18d the deletion is as indicated. In pMS1.28d only 54 base pairs of the p_L region remain. G* is the 54th nucleotide of the p_L transcript, which is normally 113 nucleotides long. *b*, The plasmids pMS32.N.Sal⁻ all derive from pMS32.00, which has been described before² and contains the MS2 DNA region 1,221-2,057. The deletions are obtained through *Bal31* digestion at the opened *EcoRI* site (1,628)¹². The nomenclature for this series is pMS32.N where N is the size of the deletion^{2,12}. To interrupt the coat-gene reading frame in these clones we have filled in the unique *SalI* site (1,366) with DNA polymerase I (large fragment). This results in out-of-phase termination at position 1,415. This series of clones is called pMS32.N.Sal⁻. The constructions were confirmed by restriction analysis and the loss of coat protein synthesis. Lysis phenotypes of all clones were determined as for Fig. 4. Sequences were determined as in ref. 16. The lysis gene is underlined and starts at nucleotide 1,678. SD is the Shine-Dalgarno sequence.

Clone no.	Lysis phenotype	MS2				
		p_L	1640	1660	SD	1680
<i>a</i> pMS1.00	-	pppAUC UUGGAAUCCGACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .				
pMS1.4	±	pppAUC UUGGAAUCC	GCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS1.6	+	pppAUC UUGGAAUCC	GAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS1.12	+	pppAUC UUGGAAUCC		AUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.17	+	pppAUC UUGGAAUCC		UAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.18d	+	pppAUC UUGG		AAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.20	+	pppAUC UUGGAAUCC		GGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.21	+	pppAUC UUGGAAUCC		GCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.26	+	pppAUC UUGGAAUCC		GCAAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.28d	+	pppAUC . . . CUG*		AAGGUCUCCUAAAAGAUUGGAA . . .		
pMS1.31	-	pppAUC UUGGAAUCC		GUCUCCUAAAAGAUUGGAA . . .		
pMS1.46	-	pppAUC UUGGAAUCC				GAA . . .

Clone no.	Lysis phenotype	MS2				
		1620	1640	1660	SD	1680
<i>b</i> Wild-type sequence		 CAUCCAAUUUUCGCCAGAAUCCGACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .				
pMS32.3a.Sal ⁻	-	 CAUCCAAUUUUCGCCAGAAU . . .	ACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS32.6a.Sal ⁻	-	 CAUCCAAUUUUCGCCAG . . .	CCGACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS32.6b.Sal ⁻	-	 CAUCCAAUUUUCGCCAGC . . .	GACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS32.9.Sal ⁻	-	 CAUCCAAUUUUCGCC . . .	GACUGCGAGCUUUAUUGUUAAGGCAUUGCAAGGUCUCCUAAAAGAUUGGAA . . .			
pMS32.32.Sal ⁻	+	 CAUCCAAUUUUCGCCA . . .		AUGCAAGGUCUCCUAAAAGAUUGGAA . . .		

our observations, since removal of GACUGC is likely to destabilize the hairpin, whereas deletions extending only 5' to this sequence will leave the helix intact. However, in the absence of structural data the model must remain tentative.

The data presented here reveal that translation of the lysis message is not unconditionally dependent on the frameshift-termination-reinitiation mechanism observed in wild-type MS2 RNA². Clearly, the lysis mRNA contains adequate initiation signals, but they are not directly accessible in the native gene setting. Therefore, the essence of the peculiar L-gene expression strategy, frameshift-termination-reinitiation, is to manoeuvre a vacant ribosome into a position unattainable by conventional initiation.

There are two possible ways to achieve this. One way is by termination of an out-of-frame ribosome at ochre codon 1,652 (-1 frame). This will melt the proposed hairpin and may allow a second (vacant) ribosome to initiate at the L-gene start (1,678). This mechanism is similar to the *lacI* restarts reported by Steege¹³. Her study shows that a terminating ribosome can expose previously masked (re)initiation sites, located 20-100 nucleotides downstream from the nonsense mutation.

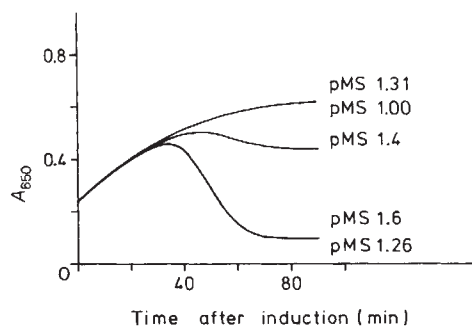


Fig. 4 Growth curves of pMS1.00 and derivatives after induction of the p_L promoter at 42°C. The various cultures are grown at 28°C until A_{650} is 0.2 and are then shifted to 42°C. In pMS1.N, the number of nucleotides deleted with respect to pMS1.00 is N.

The second way is by the +1 frameshift error which leads to termination at nucleotide 1,672 (ref. 2). This places the terminating ribosome in the initiation site. In this case the distance between stop and start codon is too small for the simultaneous presence of two ribosomes. Thus termination and reinitiation are most probably performed by a single ribosome, a mechanism which may also operate in restarts in the *rII*B gene of phage T4 (ref. 14) and in translational coupling between *trpE* and *trpD* cistrons³. In the latter two instances stop and start codons are also close together or overlapping. (This mechanism is further discussed in refs 3, 12, 14.)

Our results provide a good example of how RNA secondary structure can restrict translation of an existing template, and

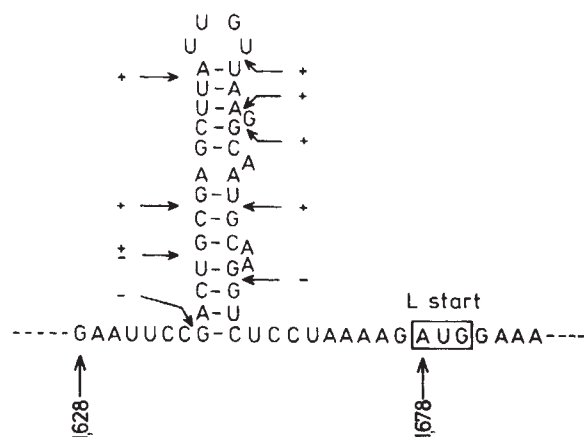


Fig. 5 Proposed secondary structure model of L-gene proximal RNA region starting at nucleotide 1,628. The AUG start codon of the L gene is boxed. Arrows indicate various positions where the p_L transcript is fused to the MS2 RNA sequence (see Fig. 3a). Lysis or the absence of lysis on induction of p_L are indicated by + or - respectively. The model proposed by Fiers *et al.*¹⁵ for this section of MS2 RNA is different and cannot explain our results.

we have suggested how such messages can be made available for translation. In the case of the L cistron the biological function of this translational control mechanism is probably to prevent premature lysis of the infected host cell.

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Cointegrates are not obligatory intermediates in transposition of Tn3 and Tn21

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The transposon Tn3 contains two genes (*tnpA* and *tnpR*) involved in its own transposition^{1–3}. The *tnpA* gene codes for 'transposase', which is absolutely required for transposition; *tnpR* codes for 'resolvase', which catalyses *recA*-independent site-specific recombination at a specific site (*res*) within the element. The usual flow diagram for transposition of Tn3 and related elements involves, first, the formation of a cointegrate of donor and recipient replicons which contains two copies of the element, and, second, resolution of this cointegrate into the original donor replicon plus the new recombinant (see Fig. 1). The formation of the cointegrate requires the transposase, and resolution is carried out by the resolvase, acting on the directly repeated copies of *res* within the elements in the cointegrate. In the absence of resolvase, cointegrates should not be resolved in a *Rec*[–] strain. Thus, if cointegrates are obligatory intermediates in transposition of Tn3 and related transposons, the sole product of transposition of a *TnpR*[–] element in a *recA* host will be cointegrates. We have examined this prediction carefully with both Tn3 and Tn21, which has analogous transposition functions to Tn3 and which is probably related to it⁴. We show here that 1–5% of transposition events involving Tn3 and Tn21 do not proceed via this intermediate. This is of significance when considering the mechanism of transposition.

The *tnpR* derivative of Tn3 that was used (Tn3.E5) was generated by insertion of an *EcoRI* linker into the *tnpR* gene^{3,5}. Transposition was from pACYC184::Tn3.E5 recombinants to pUB307 in a *recA* strain. Plasmid pUB307 is transfer-proficient, while pACYC184 is not, and is not mobilized by pUB307 (see Table 1). Thus, transposition of the Tn3 derivative to pUB307 was assayed simply by constructing a strain containing pUB307 and a pACYC184::Tn3.E5 recombinant, and then mating out

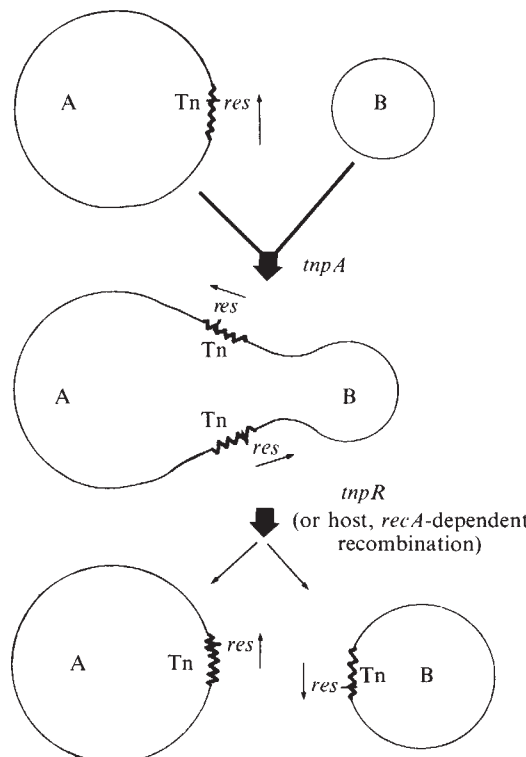


Fig. 1 Model for transposition involving a cointegrate intermediate^{1,2}. The product of the *tnpA* gene ('transposase') mediates the formation of a cointegrate of the donor replicon (A) and the recipient (B), such that they are separated by directly repeated copies of the element (Tn). Resolution of the cointegrate then restores A and gives a B::Tn recombinant. Resolution results from recombination between the directly repeated copies of the element, either by homologous recombination mediated by the host's own recombination system, or by site-specific recombination at *res* mediated by the *tnpR* gene product ('resolvase').

and selecting for penicillin-resistant (Ap^r) transconjugants. All, of course, contained pUB307 markers [tetracycline resistance (Tc^r) and kanamycin resistance (Km^r)] and most were also resistant to chloramphenicol (Cm^r) (Table 1). As Cm^r is one of the markers on pACYC184 (and is not on pUB307) this result suggests that these transconjugants had acquired cointegrate molecules comprised of pUB307 and pACYC184::Tn3.E5. When strains containing these putative cointegrates were mated with another *recA* strain, all transconjugants selected for one of the pUB307 markers were also Cm^r. However, when a plasmid containing wild-type Tn3 (that is, *TnpR*⁺) was introduced into the strain before the mating, the linkage between Cm^r and pUB307 was lost (data not shown); such *tnpR*-dependent loss of linkage is as expected for cointegrate molecules (see Fig. 1) and the data obtained are analogous to the results of other workers².

However, in addition to these cointegrates, a small proportion of Ap^r transconjugants were Cm^s (Table 1); the Ap^r displayed by these transconjugants indicates that they should contain transposition-generated recombinants, while their lack of Cm^r shows that they cannot be cointegrates. That Cm^r transconjugants contained cointegrates, while Cm^s transconjugants contained simple pUB307::Tn3.E5 recombinants, was confirmed by analysis of the plasmid DNA (data not shown).

The experiments were repeated using the deletion derivative of Tn3 that originated on the plasmid RSF1596 (ref. 6). This deletion inactivates both the *tnpA* and *tnpR* genes and also removes the *res* site. The lesion in *tnpA* can be complemented in *trans*. When this was done, the deletion derivative transposed to pUB307 and the frequency of 'direct transposition' compared with cointegrate formation was 4/100. Thus, not only is the *tnpR* gene product not required for the generation of the final products of transposition, but the *res* site is unnecessary also.

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Table 1 Direct transposition of Tn3.E5 from pACYC184::Tn3.E5

Experiment	Plasmid donor	Cm ^s Ap ^r /Ap ^r transconjugants
1	pUB1290	1/100
2	pUB1290	4/100
3	pUB1290	5/100
4	pUB1292	11/100
5	pUB1292	2/100

Strains of UB5201 (*met pro gyrA recA* 56)⁴ containing pUB307 (a derivative of RP1 lacking TnA; it codes for Tc^r and Km^r)¹⁰ and either pUB1290 or pUB1292 (see below) were mated with UB1637 (*his lys trp rpsL recA* 56)⁴ and Ap^r transconjugants selected on nutrient agar containing carbenicillin (500 µg ml⁻¹) plus streptomycin (200 µg ml⁻¹). 100 Ap^r transconjugants were then tested for Cm^r and for pUB307 markers: all contained the latter. (The transfer frequency of pUB307 (Km^r transconjugants/donor) was ~10⁻², and the transposition frequency (Ap^r transconjugants/Km^r transconjugants) was 10⁻²–10⁻¹.) pUB1290 and pUB1292 are different pACYC184 (*tet*::Tn3.E5) recombinants (P.M.B., unpublished), which code for Cm^r and Ap^r. When strains containing the parent plasmid pACYC184 and pUB307 were mated with UB1637, Cm^r was not transferred (frequency of <10⁻⁷ that of transfer of Km^r), thus pUB307 does not mobilize pACYC184 at a significant frequency. Cm^sAp^r transconjugants result from the transfer of simple pUB307::Tn3.E5 recombinants (that is, the products of direct transposition), while the Cm^rAp^r transconjugants result from the mobilization of the pACYC184 moiety as a cointegrate with pUB307 (see text).

We do not doubt, therefore, that some of the recombinants isolated were not cointegrates, despite the *tnpR* character of the Tn3 derivative. It is possible that these recombinants arose by deletion of cointegrates (for example, if the *recA* strain used was leaky, the cointegrate would be resolved by homologous recombination). This is ruled out by the observation that cointegrates from experiments such as those in Table 1 were completely stable for several months in the *recA* strains used: Km^r remained linked to Cm^r, and analysis of plasmid DNA confirmed that the cointegrates remained intact (data not shown). (In the equivalent Rec⁺ strain, however, resolution of these cointegrates did occur (data not shown)). Such stability of cointegrates in *recA* strains and their resolution in Rec⁺ strains has been well documented previously^{2,7}. Furthermore, analysis of some of these recombinants showed that their sizes were exactly as expected for a simple insertion of Tn3 (which is 4.957 kilobases, kb³); thus, for example, in one of the recombinants, the only difference between its *Sst*II fragment pattern and that of pUB307 is a change in an *Sst*II fragment of 1.8 kb in pUB307 to one of 6.8 kb in the recombinant. That deletion should give precisely the expected sizes in several different recombinants seems highly unlikely. Therefore, we believe that the plasmids in the Cm^s transconjugants from the experiment described in Table 1 were not derived from cointegrates. Accordingly, these recombinants must have arisen by 'direct transposition', that is, transposition that does not involve a cointegrate intermediate.

Next we determined whether these products of 'direct transposition' still contained a fully functional transposable element. pUB307::Tn3.E5 plasmids from Cm^s transconjugants isolated from the experiment shown in Table 1 were introduced (by conjugation) into UB5201 carrying pACYC184. These strains were then mated with another *recA* strain (UB1637) and Cm^r transconjugants selected. As pACYC184 is not mobilized by pUB307 (see above), mobilization in this case must be due to transposition of the Tn3 derivative to pACYC184, followed by transfer of the resulting cointegrate. Such cointegrates were then transferred to a Rec⁺ strain where they resolved, giving pACYC184::Tn3.E5 recombinant plasmids which were recovered by transformation. Such recombinants were then used to repeat the experiment shown in Table 1, with essentially identical results (see Table 2). Thus, the element in the recombinant plasmids that was formed by direct transposition of Tn3.E5 transposed, first back to pACYC184, and from there

Table 2 Transposition of Tn3.E5 from recombinants generated in experiments in Table 1

Plasmid donor	Cm ^s Ap ^r /Ap ^r transconjugants
pUB2187	3/100
pUB2188	2/100
pUB2189	3/100
pUB2190	3/100

Derivatives of UB5201 containing pUB307 and one of the donor plasmids were mated with UB1637 and transconjugants selected and screened as described in Table 1. pUB2187, pUB2188 and pUB2189 are pACYC184::Tn3.E5 recombinants derived by transposition from different pUB307::Tn3.E5 recombinant plasmids generated by direct transposition; pUB2190 was derived by transposition from a molecule that was originally a cointegrate (see text). All code for Cm^r, Tc^r and Ap^r. As in Table 1, Cm^s transconjugants contain the products of direct transposition, and Cm^r transconjugants contain cointegrates.

to pUB307; furthermore, transposition of this element still gave predominantly cointegrates. As a control, a cointegrate from a Cm^r transconjugant from the experiment in Table 1 was resolved (in a Rec⁺ strain) and the element on the resulting pUB307::Tn3.E5 recombinant was taken through the same procedure; this gave Cm^s transconjugants (that is, cells containing the products of direct transposition—see Table 2). Therefore, the different products of transposition in these experiments involve the same element and were not due to a heterogeneous Tn3 population: any Tn3.E5 gave both types of recombinant.

Essentially identical experiments have been performed with a TnpR⁻ derivative of Tn21 (data not shown). In this case, R388 was used as the conjugative plasmid instead of pUB307, and direct transposition was observed using either pACYC184 or pBR322 derivatives as transposition donors. Thus, the identity of the donor and recipient replicons in transposition is irrelevant in determining whether direct transposition will occur.

Hence, transposition of Tn3 and Tn21 does not involve an obligatory cointegrate intermediate. Note that this conclusion is not actually at variance with the results of others: it is just that they have ignored events that happened at low frequency. Gill *et al.*⁷, for example, showed in their Table 3 that transposition of a TnpR⁻ derivative of Tn3 generated cointegrates in 39 of the 40 recombinants examined. This agrees well with the results presented here.

Direct transposition cannot proceed by the mechanisms proposed by Arthur and Sherratt² and by Shapiro¹: these stipulate obligatory cointegrate intermediates. However, the mechanisms proposed by Galas and Chandler⁸ and by Harshey and Bukhari⁹ do account for both cointegrate formation and direct transposition; which route is taken depends on which of two strands is finally cut. Thus, for direct transposition, the 5' strand of the inverted repeat would have to be cut at one end of the element and the 3' strand at the other and (that is, both cuts would be in the same DNA strand of the element). We suggest that the transposition apparatus of Tn3 and Tn21 can cut either strand at the ends of the element, but that one is cut more efficiently than the other, so that both cointegrates and direct recombinants would be produced, but at different frequencies.

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Strands of science

A. Rupert Hall

Science Deified & Science Defied: The Historical Significance of Science in Western Culture from the Bronze Age to the Beginning of the Modern Era c.BC 350-AD 1640.

By Richard Olson.

University of California Press: 1982. Pp.326. Hbk \$32.50, £27.50; pbk \$9.95.

ON contemplating the course of science in the twentieth century many people are prone to utter cries of alarm about the culture of the future, and to accept any discussion of the distinction between the scientific and the literary or artistic frame of mind as betokening a destruction by science of 'traditional human goals and values'. Quoting David Hume's famous *mot* about the virtue of destroying books that contain neither mathematics nor experiments (but omitting to note that Hume was not a scientist, being a philosopher and historian) Richard Olson takes this fearfulness as a starting-point and as a reason for examining the interrelations between science and man's other pursuits from agriculture to theology. This volume ends with Francis Bacon, another is to follow.

He deserves credit for offering a definition of science: "a set of activities and habits of mind aimed at contributing to an organized, universally valid, and testable body of knowledge about phenomena". This is to say, science and 'doing science' are the same thing. No one would pretend that it is easy to define the nature of natural science (Mr Olson's subject) but it is a little puzzling to find the assertion, some pages later, that "the Mesopotamian scribes and astral priests... were engaged in fundamentally scientific activities."

The defect of the definition rendering such a conclusion possible lies in the adjective 'testable' which is inadequate to define the processes of verification/falsification in modern science. It is unlikely that the Babylonians made systematic tests of the accuracy of their astrological predictions; on the other hand, their astronomical computations (which, Neugebauer claims, have no connection with astrology) were self-testing.

It is less surprising to read subsequently that "there is no defining characteristic of pre-Socratic philosophy that is not included among those of science" and to find Plato and Aristotle treated as ancient 'scientists'. The early Fathers of the Church to whom Mr Olson next turns (a pity, for it would have been interesting to hear about the relation of science to the practical achievements of the Romans) were distrustful, or at least barely tolerant, of the study of nature; they were perhaps more influential than Mr Olson allows in first establishing Aristotle as the paragon of pagan learning.

From them a well-trodden path of intellectual history is followed through the universities, the Nominalists and the Humanists to the scientific renaissance. Here the positive interaction of interest is that between learning and architecture whereas the ancient world had been characterized by a negative interaction between learning and religion. In the last chapters the origins of the "secular scientism" of Francis Bacon are traced through neo-Platonism, magic and utopianism.

Mr Olson argues clearly and has made good use of his authorities and sources. If less novel than he may suppose his book is serious (as befits its academic origins) and provides a useful introduction to problems of historical understanding. There are

many consistent misspellings of names and some errors: the caption on p.217 is wrong (Theodoric taught that the primary rainbow arises from one internal reflection within the raindrop, the upper secondary bow from two internal reflections) and (p.219) Brunelleschi must have painted a correct *mirror-image* picture for his device to work. It is curious to say that renaissance engineers had "deeply imbibed scientific attitudes" and then cite the Marchese Guidobaldo del Monte (a mathematician) as an example. And so on.

For myself, I cannot believe it promotes historical understanding to refuse, as Mr Olson does, to distinguish between philosophy, science and technology; nor am I convinced by him that there is continuous strand of "scientism" running through history, which was sceptical in Antiquity but "humane and sacred" during the Renaissance. The transformation of this tradition into "the 'objective' anti-humanistic and amoral scientism of [Daniel] Defoe and [Emil Du Bois] Reymond" will be the subject of Mr Olson's second volume. □

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Cause for thought

Martin Fransman

Explaining Technical Change: A Case Study in the Philosophy of Science.

By Jon Elster.

Cambridge University Press: 1983.

Pp.268. Hbk £22.50, \$39.50;

pbk £7.50, \$11.95.

WHILE the study of the determinants of technological change is at least as old as the discipline of political economy, relatively little progress has been made in illuminating the causes of such change. This lack of understanding is evident in the title of a recent book by one of the foremost authorities in the area of technology, Nathan Rosenberg: *Inside The Black Box: Technology and Economics* (Cambridge University Press, 1982) and in the conclusions of a recent review of literature by Kamien and Schwartz (*Market Structure and Innovation*, Cambridge University Press, 1982). In view of the importance of technological change, a book entitled *Explaining Technical Change* is welcome.

One of the principal strengths of this book is that it serves to make those interested in understanding technological change more aware of what is required in order to provide a rigorous explanation of the phenomenon. This is important because the methodological underpinnings of economic models are all too often implicit and inadequately examined. In the first part of the book, the contrast is drawn

between different forms of explanation in the physical, biological and social sciences: namely causal, functional and intentional explanations. The second section provides a useful summary of the analyses of technological change that have taken place from Marx and Schumpeter, to more recent writers such as Nelson and Winter and many readers will find this of value. Particular attention is paid to the determinants of the rate and direction of technological change.

The main problem with the book, however, is that while it does provide some critical and at times insightful comment relating to the more general questions of explanation raised in Part I, it fails to go beyond the views of the authors discussed and therefore leaves the reader with a number of important questions unanswered. For example, the scope that the author sees for causal and functional explanations of technical change is, in the end, unclear although he believes that intentional explanations taking account of uncertainty have an important contribution to make.

Similarly, it is unclear what judgment the author finally makes about the value of many of the contributions that he discusses. While we are told, for example, that there is a "serious inconsistency in Marx's thought" (p.184) Elster does not round off the discussion with an assessment of the contribution that Marx made, or failed to make, to the attempt to understand technical change. A concluding chapter which tied together these and other issues would have been most

helpful. While many of the conclusions that the author arrives at regarding particular schools of thought or contributions are unexceptional (for example in the case of the neo-classical economics approach), a concluding chapter could have strengthened the link with the earlier discussion of forms of explanation.

Finally, since the author states that his "personal interest and competence are higher" (p.92) in the area of Marxist theories of technical change than in the other areas, it is worth making two rather important observations about Marx's views. The first is that although Elster suggests to the contrary, Marx was aware of the importance of capital-saving technological change and indeed as Rosenberg shows in his book, Marx elaborated on the

importance of this form of change in the machine producing sector. Secondly, it is surprising, in view of Elster's predilection for intentional explanations, that he does not discuss Marx's oft-repeated statement about social forces operating "behind the backs of individual capitalists and independently of their will". Clearly, this is of central importance in Marx's discussion of technological change and although there are connections here with Elster's discussion of functional explanations, he does not consider this issue at all.

It is a very stimulating book however, which will, I am sure, be of interest beyond a specialized readership. □

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Brackish communities

J.D. Burton and M. Sheader

Estuaries and Enclosed Seas.

Edited by Bostwick H. Ketchum.

Elsevier: 1983. Pp.500. Dfl.400.00, \$170.25.

ALTHOUGH numerous accounts of individual estuarine systems and coastal sea areas have been published in recent years there have been fewer attempts to synthesize the available information into accounts of how these environments function as ecosystems. The present volume, Number 26 in the series *Ecosystems of the World*, consists of two sections of about equal length. The first, which deals with estuaries, is structured around treatments of particular groups of organisms, drawing upon information on various localities to exemplify the processes discussed. This structure provides a better framework for integration than that adopted in Section II, which is organized on a regional basis, with accounts of ten enclosed seas. Although enclosed seas often possess estuarine characteristics on a large scale, this theme is not consistently followed and the combination of these sections in a single volume is justified in practice on grounds that are more geographical than ecological.

There is, however, a more integrated overview of the characteristics of these inland seas, provided by Bostwick H. Ketchum, the editor of the volume. Sadly, Dr. Ketchum, whose thoughtful contributions here are typical of his publications on estuaries, died before the book appeared.

The greatest single feature that distinguishes estuarine ecology from that in other aquatic environments is the intense variability of conditions. Although influenced by topography, most strikingly apparent are the marked changes in salinity distri-

butions during the tidal cycle and with season. Some background knowledge of physical processes is essential to an understanding of estuarine biology and it is provided here by chapters on estuarine characteristics and the physics of circulation. The latter, by C.B. Officer, provides a remarkably clear picture without demanding much mathematical insight by the reader, and deserves to be widely read. In contrast with the care taken to provide this background, there are no general accounts of estuarine sedimentology and chemistry. These are surprising omissions, especially since it is characteristic of estuaries that there is usually a close coupling of processes in the bottom sediments with those in the water column, with important consequences for the cycling of organic material and nutrients.

The temporal variability in conditions encountered in estuaries means that organism populations need to be adapted to stress, and a chapter is devoted to this topic. The assessment of stress in dynamic systems which are also subject to pollutant inputs is a complex question, central to evaluating the capacity of a system to receive waste without incurring unacceptable effects. The presentation here deals with the background clearly. A feature throughout the book, however, is that the literature after 1977 is thinly represented. A consequence in this particular chapter is that ideas concerning biochemical and cytological indices of stress are neglected.

The estuarine organisms are considered in separate chapters; phytoplankton, zooplankton, benthos, and fishes. The authors have adopted different approaches, each of which is successful within the limits imposed by this structure. For example the distributions of zooplankton are considered by reference to several specific regions and their general biology by reference to the copepods. The chapter on benthos is more general, with an emphasis on European estuaries. One consequence of the variability of estuaries is that they have considerable individuality, making

generalizations difficult, especially as at present we know much more about the estuaries, often small, in developed, mostly temperate regions, than those elsewhere.

A more avoidable limitation in this section arises because it tends to treat the estuarine biota in compartments. One result is that the meroplankton receive less prominence than seems appropriate. The authors do provide some information on interrelationships between the groups but there is no overall discussion, taking a holistic view and drawing on experience with estuarine ecosystem models. This would have highlighted important topics which are inadequately represented as it stands, including the function of heterotrophic micro-organisms in the cycling of organic material, and the roles of the microplankton and the meiofauna in food-chain dynamics. At the other end of the size distribution the macroalgae and higher plants receive little attention.

The chapters on enclosed seas vary in emphasis. They cover the Mediterranean, Black, Red, Baltic, Bering, Okhotsk, Japan and China Seas, and the Gulfs of St. Lawrence and California. The account of the Black Sea, by Yu. I. Sorokin, discusses succinctly the relationships between microbial activity and chemical conditions, which in turn are related to topography and water circulation. The other accounts give far more attention to environmental characteristics than to the biology. That on the Red Sea is at the extreme, with only six sentences on the organism populations.

It is useful to have these accounts brought together in one place and those for regions which have not been extensively reviewed elsewhere are particularly welcome, but generally there is surprisingly little about the inter-relationships between the organisms and their environment. Indeed for the Mediterranean Sea the biological and physical aspects are segregated into two chapters.

To make a conceptual synthesis of material concerning environments of such great diversity represents a major challenge. This volume succeeds in bringing together a large amount of well-indexed factual information but the lack of a consistently process-orientated ecological theme running through all the contributions is apparent. □

J.D. Burton and M. Sheader are in the Department of Oceanography at the University of Southampton.

Charting the Oceans

The Times Atlas of the Oceans, published by Times Books/Van Nostrand Reinhold (£30, \$80.50) is now available. Detailed maps cover the oceans of the world but a large part of the book is devoted to the marine environment. Extensive illustrated descriptions of the distribution and exploitation of marine resources are included.

Tackling autism

P.E. Bryant

Autistic Children: New Hope for a Cure.

By Niko Tinbergen and Elisabeth A.

Tinbergen.

George Allen & Unwin: 1983. Pp.362.

£19.50, \$39.50.

THERE is nothing to be surprised at when people disagree, and disagree very strongly, over questions of psychology. But sometimes our controversies get out of hand, as, I am sorry to say, we are beginning to see in discussions of childhood autism.

Feelings run strongly about autism, partly because the condition is a very distressing one for the children and their families, and partly because there is a particular poignancy in the question of what it is that causes the problem. The most striking of the many difficulties which beset these unfortunate children are social ones. They avoid contact with other people; they are withdrawn; their language is usually hopelessly threadbare and imperfect. One possibility is that these problems are the result of some fault in the child's environment and in this case, almost inevitably, the parents would be to blame. But to add to the parents' already considerable burden by making them feel guilty as well is not a step to be taken lightly, and that is why several people have argued that we need some very convincing evidence before taking the environmental hypothesis at all seriously.

For some time now the strongest support for this hypothesis has come from the Tinbergens. Their idea has been that all children have conflicting feelings when they are in the company of other people and particularly when they meet strangers. Children want to make contact with these people, but fear them and want to avoid them too. Autistic children, the Tinbergens argue, also experience this approach-avoidance conflict, but in their case the tendency to avoid is usually overwhelming. What, the Tinbergens then ask, is the reason for this? For an answer they turn to the autistic child's very early social experiences and argue that these go terribly astray. They also offer a number of reasons, such as parental inexperience and the birth of a brother or sister.

These ideas led the Tinbergens to consider methods of helping autistic children. When they first published their ideas on autism over ten years ago, the Tinbergens made some interesting suggestions about ways of getting round the avoidance conflict by approaching these children indirectly: touch the child before you try to catch his eye, was their advice, and though they offered little evidence for this suggestion it seemed a simple and testable one. But they have largely abandoned this idea in their new book, in which a great deal of space is devoted to recommending methods

of treatment which certainly would have horrified the authors ten years ago. The method which impresses them most consists of the mother holding her autistic child forcibly for quite a long time and doing everything she can to meet the child's gaze. I find it difficult to understand why on the Tinbergens' own hypothesis this would not increase rather than decrease the conflict which they think so crucial to autism. But the treatment concentrates on building up the bond between parent and child and thus fits in with their causal ideas.

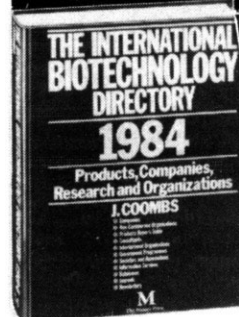
Are these ideas justified? One of the most striking features of this book is that it offers no new systematic positive evidence at all. Time and again the Tinbergens appeal to their experience with individual children whose case histories fit their theory (the data are described as "incidental"). As often they cite the successes of various methods of treatment which centre around building a proper relationship between autistic children and their parents. Both kinds of evidence are plainly inadequate: there is no proper comparison here with other groups of children or with other types of treatment.

The authors deal in impressions, and their confidence about doing things in this way is evidently bolstered by their scorn for the evidence offered by people who hold contrary views to their own. It is here that the argument takes a sorry turn. The Tinbergens' opponents are constantly described as "experts", a term which becomes overtly pejorative when we are reminded how ignorant experts can be. The research of these experts — who are said to stay mainly in their offices and deal in standard and thus insensitive tests, and who are even criticized for writing prolifically — is described summarily and sometimes incorrectly. For example, one of the few good studies of the effects of different methods of teaching autistic children — carried out by Bartak and several colleagues — is described quite wrongly as showing no difference between the various regimes: on the contrary the differences were very great and went, it seems to me, in the opposite direction to the Tinbergens' hypothesis.

At the moment it is quite impossible to know to what extent the Tinbergens or the "scientists" and "doctors", "child psychiatrists" and "experts" whom they reject are right. It seems to me that the Tinbergens' plea for more research using informal observation is justified, although so far very little of substance has come from it. Their new book does nothing to establish their own hypothesis and a great deal to lower the tone of the discussion of childhood autism. But if someone now begins a proper evaluation of the methods of treatment which the Tinbergens describe in their book, some good might come from it for the autistic child. □

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Astronomy

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